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- P. 345, line 3, for "var. *williamsonii*" read "var. *congdonii*"
 P. 349, line 21, for "*luteola* var. nov." read "var. *luteola* var. nov."
 P. 362, line 18, for "retrosum" read "retrorsum"
 P. 364, line 28, for "retrosum" read "retrorsum"
 P. 367, line 3, for "margini" read "margine"
 P. 371, line 11, for "**americanorum**" read "**americanarum**"
 P. 373, line 8, for "**americanorum**" read "**americanarum**"

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- P. 252, line 20, for "(1)" read "(1a)"
 P. 304, line 6, delete parenthesis and precede by "as in"

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PROTHALLIA OF THE CYATHEACEAE

ALMA G. STOKEY

(WITH ONE HUNDRED AND EIGHTY-SIX FIGURES)

Introduction

The family Cyatheaceae is listed by CHRISTENSEN (7) as consisting of seven genera and 456 species. Of these, three genera and 426 species belong to the Cyathea, one monotypic genus to the Thyrsopterideae, and three genera and twenty-nine species to the Dicksonieae. Scarcely twenty species have been described in the gametophytic stage, and about half of these have been studied only in the germination stage or have been the subject of experiments in germination. BAUKE (1) gave a complete history of the gametophyte, based on a study of *Cyathea medullaris*, *Alsophila australis*, and *Hemitelia spectabilis*, with a less complete study of *Balantium antarcticum*, *Cibotium schiedii*, a second *Alsophila*, and a second *Cyathea* species. WIGAND (30) in 1854 gave an account of the antheridium and noted the multicellular hairs on the prothallium. KNY (15) reported on the germination, development of prothallium, and structure of the antheridium of *Cibotium schiedii*. HEALD (12), SCHULTZ (24), LAAGE (16), and LIFE (18) gave accounts of the relation of light to germination of spores, based in part on species of the Cyatheaceae. HEIM (13) reported on some experiments on the influence of light on the formation of sex organs, using among other species two of the Cyatheaceae, *A. australis* and *Balantium antarcticum*. HEIM also discussed the characters of the gametophytic stage

and their value in classification. GOEBEL (11) discussed the prothallia of *B. antarcticum*, *Hemitelia gigantea*, and *H. walkerae*, particularly with reference to the production of multicellular hairs and the branching of the prothallia. SCHLUMBERGER (23) gave an account of a study of the antheridium of three species of the Cyatheaceae (*Cyathea dealbata*, *Hemitelia aspera*, and *Cibotium schiedii*), and made a comparison of the antheridium and multicellular hairs of the Cyatheaceae with those of certain species of the Polypodiaceae. STEPHENSON (25) reported on young stages and the antheridia of *Dicksonia squarrosa*, *Cyathea dealbata*, *C. medullaris*, and *C. cunninghamii*.

The present paper is the result of an attempt to make a comprehensive study of representative species of the Cyatheaceae, to ascertain the range of type within the family and to obtain light on the origin of the family. The similarity of the gametophyte to that of the Polypodiaceae has probably received sufficient emphasis, but the similarity to more primitive groups has received relatively little. The work was begun before the appearance of the paper by BOWER (4) in 1913, presenting the theory that the family is polyphyletic and consists of representatives of two distinct lines, the Superficiales and the Marginales, which have reached approximately the same stage in evolution. In the light of this theory it has been particularly interesting to note to what extent the evidence given by the gametophyte suggests a polyphyletic origin.

This work has been carried on over a period of eighteen years, and the results are based on more than sixty cultures, each from a different collection of spores. Each culture was carried for at least four months, most of them from eight months to a year, several for two years, and two for five years. All species were brought to the stage of the production of archegonia and sporelings. For the sake of comparison, cultures were made of representatives of the Osmundaceae, Gleicheniaceae, Schizaeaceae, and Polypodiaceae.

The spores used for culture were obtained from many sources. The writer wishes to express her indebtedness to the following: Dr. N. L. BRITTON and Dr. M. A. HOWE of the New York Botanical Garden; Mr. L. A. BOODLE of the Royal Botanic Gardens, Kew; the Director of the Botanic Gardens, Edinburgh; the Director of

Horticultural Hall, Fairmount Park, Philadelphia; Dr. F. E. STOKEY for collections in Madeira; Mr. OTTO DEGENER for collections in the Hawaiian Islands; Mr. D. WATT for collections in Jamaica; and also to Mr. C. A. WETHERBY of the Gray Herbarium, Cambridge, for the determination of *Cibotium barometz*; and to Dr. W. R. MAXON of the National Herbarium, Washington, for the determination of all other species used in this investigation.

Method

In raising fern prothallia it is necessary to take precautions to obtain pure cultures. Naturally it is necessary to avoid contamination by fungi and algae, both of which are apt to flourish under conditions which are favorable to fern prothallia. It is equally important to avoid the introduction of the spores of other species of ferns. In greenhouse material especially, one is apt to find spores of other ferns deposited on the leaves, and unless these are removed it may happen that the prothallia of the desired species will be crowded out by the more rapidly growing prothallia of undesired species; but of course the chief objection is the inability to separate a mixed culture until it is too late. In the case of greenhouse material a method referred to in an earlier paper (27) was found satisfactory. Portions of fertile leaves were collected when the sporangia were ripe but had not yet opened; each portion was carefully brushed with a camel's hair-brush while under running water. Few, if any, foreign spores will remain if this process is carried out thoroughly. Even after these precautions are taken it is desirable to have some checks on the purity of the cultures. The spore coat remains attached to the young prothallium for a considerable time, and this is an aid in judging the purity of young cultures. The uniformity shown by a culture in its rate of growth and development is a good indication of its purity. The real test, of course, is the production of mature sporophytes true to type, but the production of a considerable number of uniform sporelings is also a good criterion. While not all the cultures discussed in this paper were carried to the mature stage of the sporophyte, they were in enough cases to warrant the assurance that the results are based on pure cultures. All the cultures of the first two years were subsequently duplicated.

It seems probable that some of the lack of harmony in the results of work on fern prothallia may be due to the use of cultures derived from greenhouse material, and that in these cases there was not sufficient care taken to avoid the introduction of foreign spores. It is possible that some may be due to error in identification of the material from which the culture was derived. Some of the difficulty may be due to lack of uniformity in methods of culture and the absence of a definite test as to what constitutes favorable or normal conditions for growth.

Germination stages were obtained on distilled water, on tap water, on porous crock standing in water, on peat, and on soil (a mixture of leaf mold and sand). The same types were produced in all cultures but the cells were usually longer in water cultures; the water culture is satisfactory for the earliest stages only. The peat cultures were sometimes slower than those of the soil, but the same type of prothallium was produced on both. A comparison of the results led after a time to the use of peat exclusively for the later stages. Peat cultures require less frequent attention than soil cultures, as peat retains water longer and stays more uniformly moist. The prothallia are cleaner and accordingly easier to handle. The peat used was for the most part black Nantucket peat which is rather hard and compact, but some of the later cultures were made on Wisconsin peat which is lighter and looser. The peat is improved by being boiled eight or ten times with changes of water. No experiments were made to determine the most favorable degree of acidity, but many prothallia were found to grow satisfactorily in peat which had a pH of 4.6.

The prothallia were raised in low stender dishes or crystallizing dishes 6-12 cm. in diameter and 3-5 cm. high, with a layer of peat 1-2 cm. deep. Such a culture does not require watering more than once in three or four weeks. The ordinary flat stender cover is not satisfactory as it is too easily dislodged; the overlapping type is better, and it was found that one plate of a petri dish of an appropriate size made a very convenient cover. Boiled tapwater was used for watering, as it was found that boiling the water reduced the danger of infection from algae. Cultures which were infected by fungi were treated with potassium permanganate. At first this

was carefully measured according to the proportions given by TWISS (28), 0.15 gm. in 3.5 liters of water, as recommended by LANG, but equally good results were obtained by using the "solution decidedly pink" to which she refers. In cases of bad fungal infection the prothallia were washed in the solution, transferred to freshly sterilized dishes of peat, and given a daily application of the solution until either the fungus or the prothallia died. It is easier to get rid of fungi than of algae. Rinsing the prothallia in boiled water and transferring them to fresh peat often enables the prothallia to keep ahead of the algae but it does not effect a cure.

The cultures were kept in a laboratory in which the temperature ranged from 17° to 24° C. They were kept near a north window where they received the maximum amount of diffused light available. Direct sunlight was found to be unfavorable. Prothallia which were kept in a shaded window or at a distance of 2 or 3 m. from the window showed many irregularities, and did not follow what may be called a normal course of development. In this paper conditions which resulted in a rapid development of the typical heart-shaped prothallium, with an early formation of the cushion and of archegonia are referred to as favorable. Conditions which caused the development of ameristic prothallia, elongated pale prothallia, prothallia which never progressed beyond the antheridial stage, or prothallia which reverted to the antheridial stage after having initiated the production of archegonia are referred to as unfavorable, since such conditions would not lead to the production of sporophytes.

Paraffin sections were made of all species studied. Several killing and fixing agents were tried, but formalin alcohol (6 cc. commercial formalin in 94 cc. 50 per cent alcohol) was on the whole the most satisfactory, although good results were obtained in some cases with a weak solution of chromo-acetic acid (1 gm. chromic acid, 3 cc. acetic acid, 300 cc. water). The stains used were Flemming's triple stain, Haidenhain's iron-alum haematoxylin counterstained with orange G, and a combination of safranin and anilin blue.

For the study of antheridia fresh material was found the most satisfactory, although in some cases it was helpful to use material which had been bleached in a weak solution of potassium hydroxide.

Cyatheae

The following list gives the fifteen species of the Cyatheae investigated, together with the length of time each was kept in culture.

Lophosoria quadripinnata (Gmel.) Bower (*Alsophila quadripinnata* [Gmel.] C. Chr.¹) 10 months, 5 years

Alsophila armata (Sw.) Pr. 10 months

Alsophila aspera (L.) R. Br. 1 year, 15 months

Alsophila atrovirens (Langds. & Fisch.) Pr. 14, 15 months

Alsophila cooperi F. Muell. 9, 10, 16 months

Alsophila excelsa R. Br. 4, 10 months

Hemitelia horrida (L.) R. Br. 5, 8 months

Hemitelia parvula (Jenm.) Bak. 11 months

Hemitelia smithii (Hk. fil.) Hk. 5 months

Cyathea arborea (L.) Sm. 7 months, 1 year

Cyathea dealbata (Forst.) Sw. 1 year

Cyathea medullaris (Forst.) Sw. 10 months

Cyathea muricata Willd. 4, 6 months

Cyathea nigrescens (Hk.) J. Sm. 10 months

Cyathea tussacii Desv. 6 months

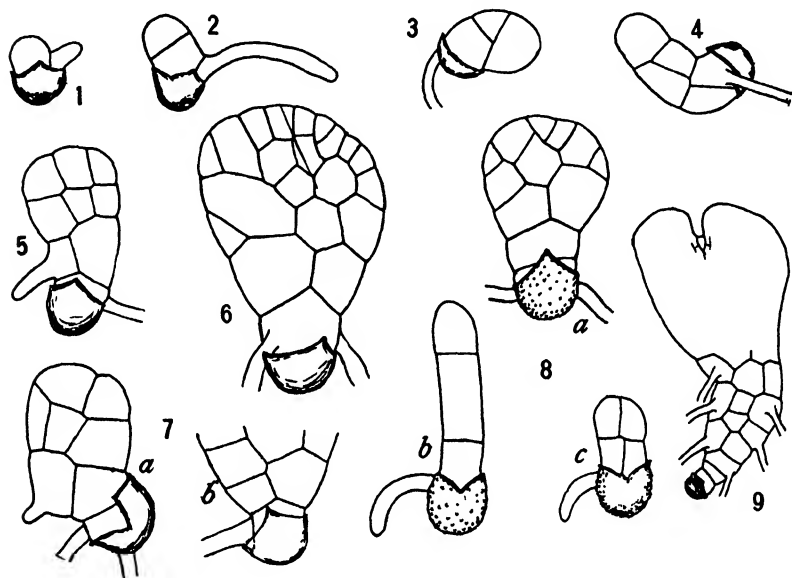
VEGETATIVE STRUCTURE

The spores of all the species studied are of the tetrahedral type but vary in size, color, and texture of wall. Some are smooth (*Alsophila excelsa*); those of *A. cooperi* are covered with spines; those of *Hemitelia horrida* have peculiar disks in the wall; while those of *Lophosoria quadripinnata* have a characteristic ridge. In general, germination was better when the spores were fresh, but in some cases a delay of 3-4 months made little or no difference; in a few cases germination was better after a resting period of several months. As a rule the percentage of viable spores decreased after 3-4 months and germination became slower. No experiments were carried on to determine how long the spores retained their viability, but in several cases it was possible to germinate spores 12-15 months old. In the case of most spores a change in color of the contents indicated that germination was about to take place, but in some

¹ In the nomenclature of this species BOWER has been followed instead of CHRISTENSEN.

cases the first indication was the rupture of the spore coat at the apex of the tripartite ridge.

The first wall is parallel to the axis of the spore and separates the first prothallial cell from the first rhizoid (fig. 1). At first the rhizoid elongates more rapidly than the prothallial cell. With the growth of the prothallial cell a wall is formed which is more or less oblique to the wall cutting off the rhizoid cell (fig. 2). The prothallial cell



FIGS. 1-9.—Figs. 1-4, *Hemitelia horrida*; figs. 5, 6, *Alsophila excelsa*; fig. 7, *Cyathea muricata*; fig. 8, *A. cooperi*, $\times 165$; fig. 9, *Lophosoria quadripinnata*, $\times 60$.

broadens as well as lengthens, pushing to one side the primary rhizoid cell (figs. 2, 7b, 8b). The next division may be a longitudinal division of the terminal cell, an oblique, or a horizontal division. Under favorable conditions the thallus broadens early; a filament of four or five vigorous cells, such as is commonly found in the Polypodiaceae, is unusual. If such a filament does form, all the cells except one (perhaps two but rarely three) at the base will divide longitudinally or obliquely at about the same time. If the culture is crowded or in weak light more or less depauperate filaments with long cells are formed (fig. 8b). Typically the only cell of

the initial filament which persists without longitudinal or oblique division is that which remains in part within the spore coat (figs. 5, 7b). Fig. 8a shows a prothallium of *Alsophila cooperi* with a 2-celled filament at the base, and fig. 9 one of *L. quadri-pinnata* with three, but it will be noted that the aspect of the prothallia is not that of the early stages of polypods. The young prothallia are distinctly shorter and broader than those of the Polypodiaceae grown under what may be called favorable conditions. The same results were obtained in many series of cultures. No notable differences were found between germination in June and December, certainly no difference which could be correlated with length of day or intensity of light. It requires a reduction below the intensity of maximum diffuse light of December in latitude 42° to produce filaments of the depauperate type. It should be noted, perhaps, that the presence of snow on the ground in Massachusetts during the winter months makes the reduction in light less than might be expected. Germination was of the same type whether on soil or peat, and for the most part whether on water or on a solid substratum. Even in the early stages, however, the water cultures were distinctly less vigorous than those on soil or peat.

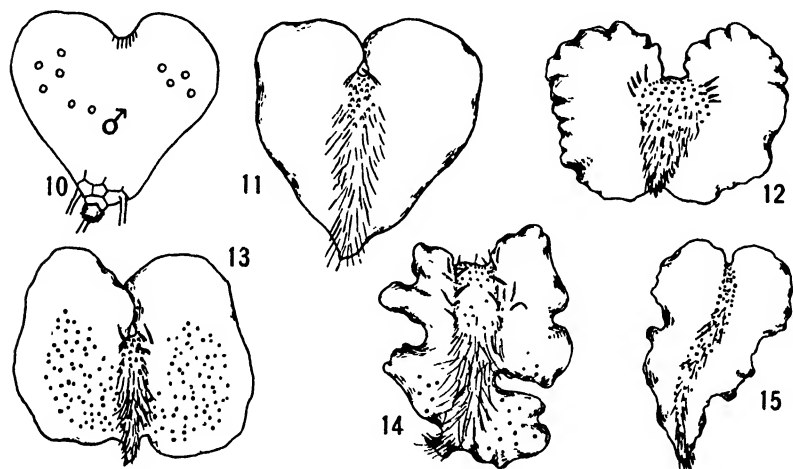
The results given here are similar to those obtained by BAUKE, who stated that the prothallia of the Cyatheaceae differ from those of the Polypodiaceae in the earlier formation of the plate and in the fact that no filament is left at the base of the prothallium.

The apical cell appears early, sometimes in the 4- or 5-celled stage but ordinarily later (fig. 8a); subsequent divisions are shown in fig. 6. The apical cell is later replaced by a group of initials. The production of antheridia usually begins about this time (fig. 10). In general the appearance of antheridia is distinctly later than in the polypods; or perhaps it is better to say that no indication was seen of the ability to produce antheridia at the very early stages (5- to 25-celled) such as is frequently found in the polypods. The cushion develops in the usual manner.

The mature prothallium of the Cyatheae is the typical heart-shaped structure such as prevails among the leptosporangiate ferns. To attain this form the prothallia must have ample space, a suitable amount of moisture, and a favorable intensity of light. While the

prothallia of the various species studied conform to the same general type, they are by no means uniform, but vary in color, size, proportion, thickness and width of rib, and amount of cutinization both on the surface and on the sex organs.

The prothallia of *Cyathea* were found to approach most closely to those of the polypods, being broader and shorter, with a smaller and thinner cushion than those of *Alsophila* and *Hemitelia*. The prothallia of *Lophosoria* were relatively thin and delicate, but are

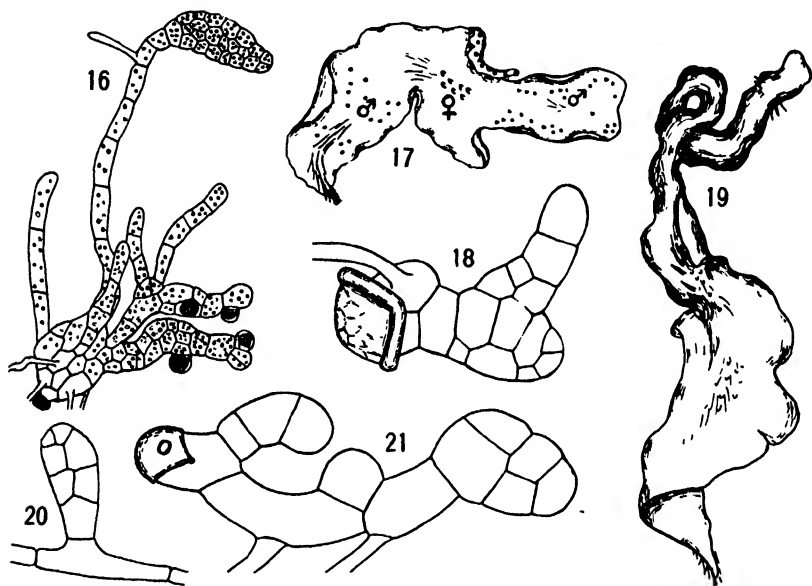


FIGS. 10-15.—Fig. 10, *Alsophila armata*, $\times 40$; fig. 11, *Cyathea tussacii*, $\times 6$; fig. 12, *Hemitelia smithii*, $\times 5$; fig. 13, *A. cooperi*, $\times 5$; fig. 14, *A. excelsa*, $\times 5$; fig. 15, *Lophosoria quadripinnata*, $\times 5$.

too long to suggest the polypod type. The prothallia of *Lophosoria* were studied in two sets of cultures, one of which ran for two years and the other for five, but at no time did the prothallia become heavy or robust in appearance. They produced healthy gametes and numerous sporelings, but they never approached the massive types found in some species of *Alsophila* and particularly in *Cibotium barometz*. Even those which developed branched cushions (fig. 24) were rather delicate. It is quite possible that the culture conditions were not so favorable for this species as for the others, although they brought the prothallia to maturity.

Forking of the apical meristem with subsequent branching of the

cushion was found to occur *L. quadripinnata*, *A. armata*, *A. atrovirens*, *H. horrida*, *H. parvula*, and *C. dealbata* (figs. 22–25). This results in two or more regions of archegonial production. The tendency to branch is shown earlier and more frequently in *Hemitelia* than in the other genera. GOEBEL (11) has discussed its occurrence in *H. gigantea* and *H. walkerae*. In some plants of *H. parvula* (fig.

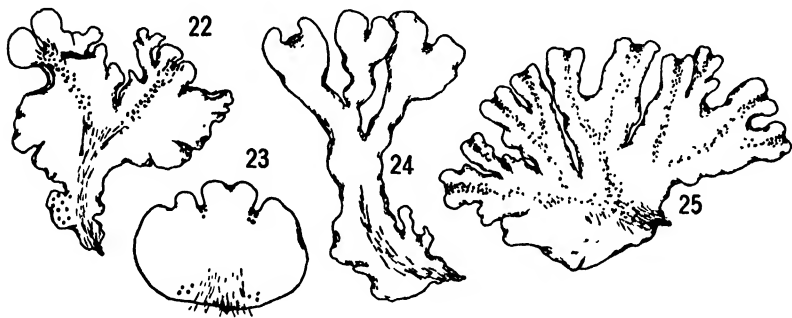


FIGS. 16–21.—Fig. 16, *Alsophila excelsa*, $\times 40$; fig. 17, *Cyathea arborea*, $\times 8$; fig. 18, *Lophosoria quadripinnata*, $\times 165$; fig. 19, *Cyathea* sp., $\times 8$; fig. 20, *A. cooperi*, $\times 80$; fig. 21, *Hemitelia horrida*, $\times 200$.

25) the branching was almost as regular as in *Riccia*, and resulted in a broad fan-shaped prothallium very suggestive of a liverwort. In *Hemitelia* forking may occur at so early a stage (fig. 23) that it seems probable that it may occur frequently in nature, but in the other forms the growth of the prothallium would ordinarily be checked by embryo formation before forking would occur.

The thickness of the cushion varies apparently both with the species and with the cultural conditions. In old prothallia which were vigorous and healthy there was a tendency for the rib to develop more strongly than the wings. In such a prothallium as that shown

in fig. 14 the wing development has not kept pace with the rib and in some prothallia the rib extends as a heavy flattened or rounded projection beyond the wings. Fig. 19 shows a 14-months old prothallium of a species of *Cyathea* from Jamaica (probably *C. serra* but the material was insufficient for positive determination) which gives such a case of rib development. The growth did not give the slightest indication of being apogamous. Such an elongated rib may become more or less erect, or in the case of more robust prothallia with a broader heavier rib, the extension may curl under or backward to such an extent that the morphologically dorsal surface be-

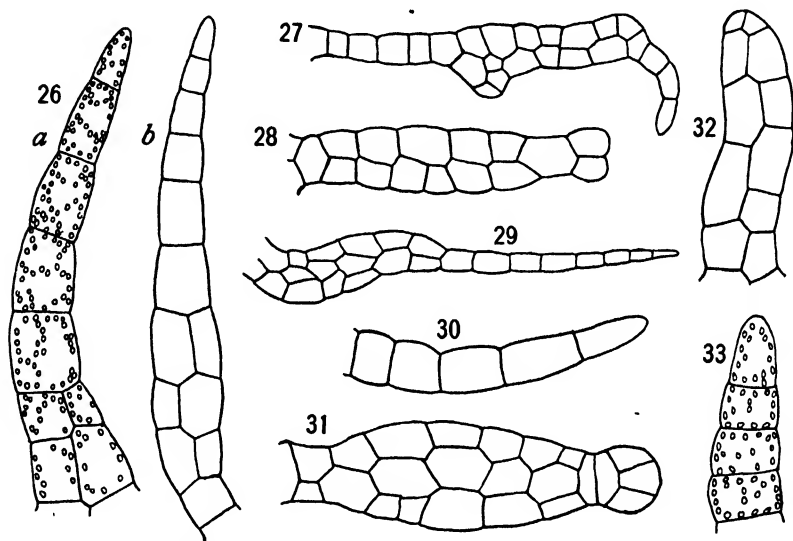


FIGS. 22-25.—Fig. 22, *Alsophila armata*, $\times 5$; fig. 23, *Hemitelia horrida*, $\times 5$; fig. 24, *Lophosoria quadripinnata*, $\times 6$; fig. 25, *H. smithii*, $\times 2.5$.

comes the physically lower, and completely takes over the formation of rhizoids and archegonia. In some cases when fertilization did not occur and the prothallia were allowed to grow indefinitely the wings tended to become irregular and were more or less curled and crisped (figs. 12, 14), suggesting somewhat the *Gleichenias* described and figured by CAMPBELL (6), but no species of the Cyatheaes included in this investigation showed such fine examples of it as did some of the Dicksonieae.

In weak light the tendency to elongate was very much pronounced, and with this there might be a noticeable change in the thickness of the thallus and then in the type of sex organ produced. Fig. 17 shows a prothallium of *Cyathea arborea* which produced antheridia, then for a period archegonia only, then antheridia only. This was found to be definitely related to the intensity of light and

consequently to nutrition, as was shown by WUIST (29) in the case of *Onoclea struthiopteris*. In certain species (*A. excelsa*) there is a strong tendency in weak light for prothallia to develop as slender branching ameristic structures which produce antheridia only, but in other species this is much less common. The branched prothallia shown in figs. 18 and 21 were from young crowded cultures. Fig. 16 shows a plate which has given rise to numerous filaments, one of which has developed a plate at its tip. In fig. 20 is shown a plate which has arisen laterally on a long filamentous prothallium.

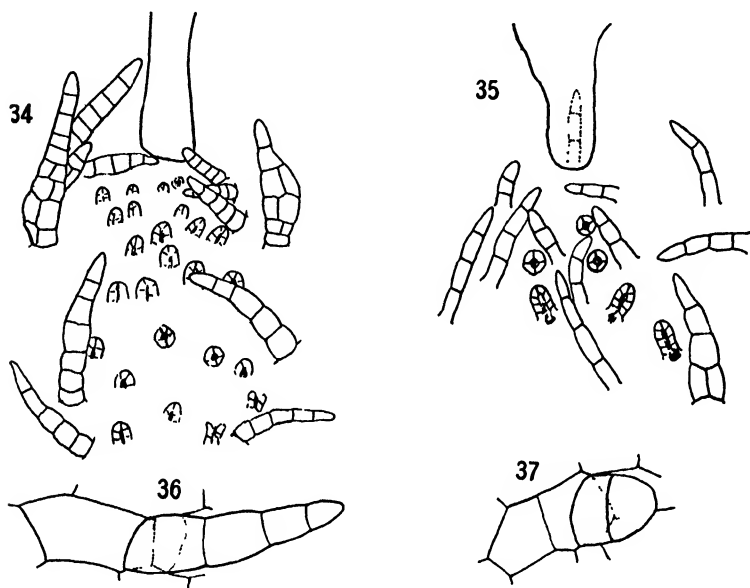


FIGS. 26-33.—Figs. 26, 28, *Alsophila excelsa*, $\times 180$; fig. 27, *Hemitelia horrida*, $\times 160$; figs. 29, 31, *A. aspera*; fig. 29, $\times 45$; fig. 31, $\times 120$; fig. 30, *Cyathea dealbata*, $\times 120$; fig. 32, *C. nigrescens*, $\times 90$; fig. 33, *A. atrovirens*, $\times 165$.

HAIRS.—References to the multicellular hairs of the Cyatheaceae are found in most of the literature dealing with mature prothallia. The hairs were first mentioned by WIGAND (30), and were later described by BAUKE (1) as “borstenförmigen Haaren.” In some accounts they are said to be characteristic of the family; in others, of a particular genus, for example, *Alsophila*. According to the present investigation they are limited to the members of the subfamily Cyathea as given by CHRISTENSEN.

The multicellular hairs show a wide range both in size and shape.

Those on young prothallia are uniseriate and may not be more than three or four cells long (fig. 33), while on old but vigorous prothallia they may be twenty to thirty cells long with an actual length of 2 mm. or more (figs. 46-48). Figs. 26, 29, 30 illustrate the most common types on prothallia 2-5 months old. The base is ordinarily more than one cell in width, but the tip is usually uniseriate and pointed; the broadened tip (figs. 28, 31, 32, 47) is relatively infre-

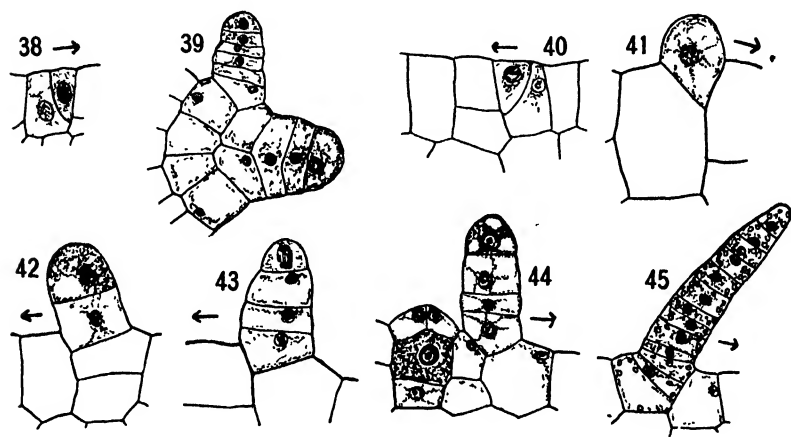


FIGS. 34-37.—Fig. 34, *Alsophila atrovirens*, $\times 48$; fig. 35, *A. aspera*, $\times 48$; fig. 36, *A. aspera*, $\times 165$; fig. 37, *A. atrovirens*, $\times 230$.

quent. Figs. 46-48 are from hairs on large prothallia. Such hairs are easily seen with the unaided eye and may give a shaggy appearance to the region of the cushion. The width of the hair usually ranges from one to three cells, but may reach eight to ten; they are ordinarily one cell thick, but large hairs may be several cells thick at the base (figs. 46, 47). All the cells of the hair may be capable of division for a considerable period. It will be seen from fig. 49 that the cells in the region above the base may persist in the meristematic condition after the cells at the base and the tip have begun to mature. In no case was a glandular tip observed nor was there any

evidence of glandular character. The cells of the hair are abundantly provided with chloroplasts and are as green as the prothallium which bears them. The cells of the prothallium at the base of the hair persist as healthy green cells.

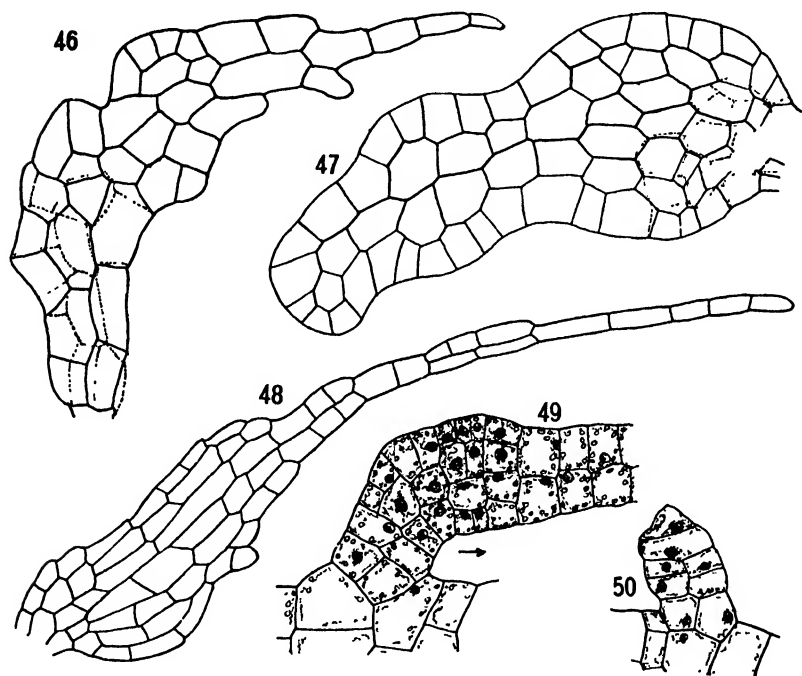
The hairs are found on both the dorsal and ventral surface of the prothallium but not on the margin or near it. On the ventral surface they usually develop along the sides of the cushion and on the adjacent regions of the wings (fig. 34), but they may be found among the archegonia (fig. 35). On the dorsal surface they are usually



FIGS. 38-45.—Figs. 38, 39, *A. armata*; fig. 38, $\times 285$; fig. 39, $\times 260$; fig. 40, *Cyathea arborea*, $\times 300$; fig. 41, *A. cooperi*, $\times 285$; fig. 42, *H. horrida*, $\times 285$; figs. 43, 44, *A. excelsa*, $\times 285$; fig. 45, *C. medullaris*, $\times 200$.

found on the cushion, spreading occasionally to the wings. The formation of hairs seems to be related to the stage of maturity and vigor of the prothallium. They do not appear ordinarily until after the formation of the cushion. In species of *Alsophila* they may appear before any archegonia have formed, but typically they do not appear until one or more archegonia have matured. In rare cases they may appear on prothallia which have not begun to thicken and which bear only antheridia. Hairs were later in appearing in *Cyathea* and *Hemitelia* than in *Alsophila*. Hairs were found on a few prothallia only in the case of *Lophosoria*; they were late in appearing, and were short and uniseriate. As noted, the prothallia of *Lophosoria*, while as long-lived as any other species in culture,

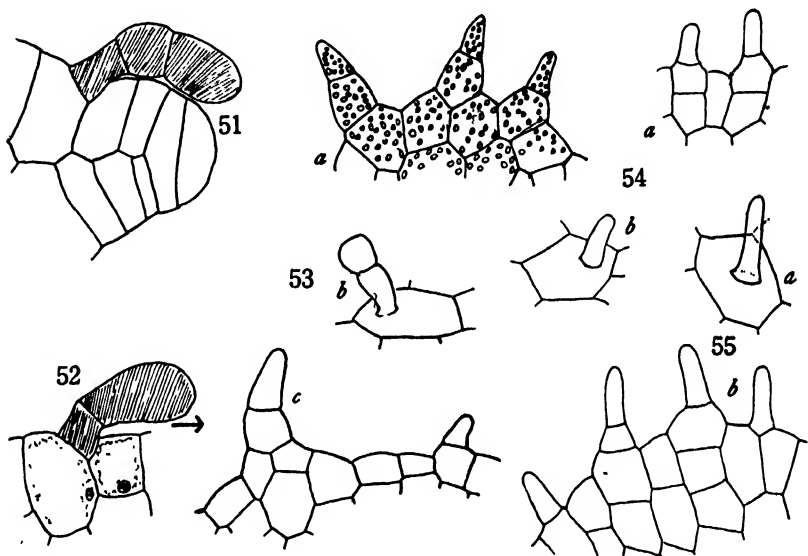
never appeared to be vigorous. It may be that the meager and belated production of hairs is to be explained in this way, but it does not seem probable. As GOEBEL (11) has pointed out, it is very likely to happen in certain species (*L. quadripinnata*, species of *Cyathea* and *Hemitelia*) that prothallia may mature, bear sporophytes, and disintegrate without having produced any hairs.



FIGS. 46-50.—Figs. 46, 47, 50, *Alsophila atrovirens*, figs. 46, 47, $\times 80$; fig. 50, $\times 210$; figs. 48, 49, *Cyathea medullaris*; fig. 48, $\times 50$; fig. 49, $\times 250$.

The origin of the multicellular hair is shown in figs. 38, 40, 41, in which the arrow indicates the position of the apical region of the prothallium. It will be seen that it arises from a wedge-shaped cell cut off from the anterior side of a prothallial cell near the apical region, being formed by a wall which is oblique to the surface of the prothallium. The later growth of the mother cell and of the adjacent prothallial cells, as well as that of the hair, gives the hair a wedge-shaped base (figs. 39, 41-45, 49). Fig. 50 shows the base of a hair

in a section cut across the cushion. Surface views of hairs arising from the thallus are shown in figs. 36, 37. This type of origin of a trichome was reported by LEAVITT (17) for the root hairs of certain species of angiosperms, and by STOKES (26) for those of certain species of *Lycopodium*. It is unlike the origin of rhizoids which form from a projecting papilla near the center of an ordinary prothallial cell. It is also distinctly different from the origin of the hairs which



FIGS. 51-55.—Figs. 51, 52, *Gleichenia pectinata*, $\times 285$; fig. 53, *Phyllitis scolopendrium*, $\times 145$; fig. 54, *Dryopteris filix mas*, $\times 145$; fig. 55, *Woodsia obtusa*, fig. 55a, $\times 165$; fig. 55b, $\times 180$.

are found on many species of the Polypodiaceae (for example, *Dryopteris filix mas*, fig. 54). Fig. 39 shows the relation of the hair to the apical cell in *A. armata*, and indicates that the wedge-shaped base is not due to a secondary division of the trichome-forming cell, as is sometimes the case in the Polypodiaceae (*Woodsia obtusa*, fig. 55b).

BECK (2) described and figured multicellular hairs on the prothallia of *Phyllitis scolopendrium* (*Scolopendrium vulgare*), comparing them with those of the Cyatheaceae. SCHLUMBERGER (23) con-

firmed this work. BECK's drawings of multicellular hairs indicate that the origin of the hair in *Phyllitis* corresponds to the usual polypod type. In the writer's cultures of *Phyllitis* the hairs present were, for the most part, of a type which indicates only a small departure from the usual polypod type (fig. 53). Very few consisted of more than two or three cells. The usual type was simple one-celled or glandular. In a species so notable for the variations of its sporophyte, however, it might well be expected that there would be considerable range in the gametophyte, and that some strains would produce better crops of hairs than others. What is more probable is that the difference is due to the fact that old prothallia were not studied. The hairs in *Phyllitis*, unlike those in the Cyathea, are found on the margin as well as widely distributed over both surfaces.

SCHLUMBERGER (23) studied the hairs of *Woodsia* and *Diacalpe*, tracing a relation between them and the type of the Cyathea, and suggested that they were a transitional form. His drawings show that in origin they are typically polypod and do not arise from a special initial. This has been confirmed in cultures of *Woodsia ilvensis* and *W. obtusa* (fig. 55).

The origin of the multicellular hairs in *Gleichenia pectinata* (figs. 51, 52) is the same as that of the hairs of the Cyathea. The hair in *Gleichenia* arises from a special initial. CAMPBELL (6) gives an illustration (fig. 107) of a hair of *G. polypodioides* which is similar to fig. 51, and indicates that the hair is formed in the same way. He also states that the hairs occur on or near the cushion among the archegonia. This is also the distribution in *G. pectinata*.

The hairs which GOEBEL (10) has described as present on the prothallia of *Loxsonia cunninghamia* are very suggestive of those of the Cyathea, both in form and distribution. Their origin is not given, and Professor GOEBEL said in a recent letter that he had unfortunately no material from which this could be determined. They conform so completely to the cyatheoid type in form and distribution that it seems highly probable that they agree in origin. This would seem to be an argument of some weight in allying *Loxsonia* with the Gleicheniaceae-Cyathea line.

REPRODUCTIVE STRUCTURES

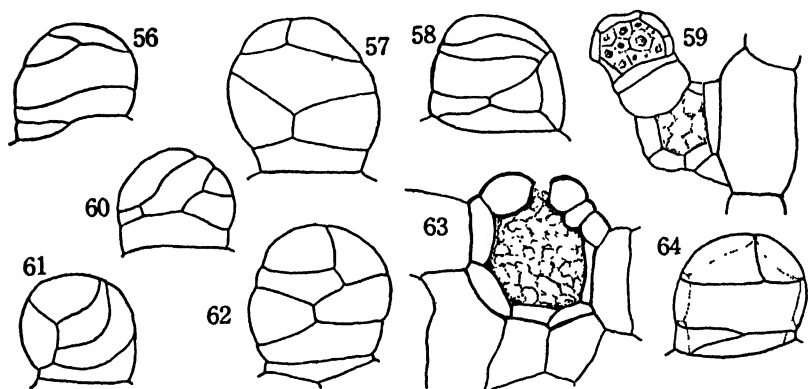
The prothallia are typically monoecious, but, as in the case of many ferns, there may be a tendency toward dioecism. In certain species, as *Hemitelia smithii*, the production of antheridia ceases when archegonia begin to appear; in others the production is more or less gradually reduced, ultimately stopping (fig. 14); in *A. cooperi* the formation of archegonia causes little or no change in antheridial production (fig. 13).

ANTHERIDIUM.—The main features of the structure of the antheridium of the Cyathea were established in 1876 by BAUKE (1), whose work was chiefly on *Cyathea medullaris*. His account, which, has since served as the basis of all general accounts of the group, was supplemented in 1907 by STEPHENSON (25), who had investigated the antheridia of *C. dealbata*, *C. cunninghamia*, *C. medullaris*, and *Dicksonia squarrosa*, and described them as being similar to the "complex normal type" described by BAUKE and showing the same variety of reduction forms. SCHLUMBERGER, in his study of transition forms between the Cyatheaaceae and the Polypodiaceae, investigated *Cyathea dealbata*, *H. aspera*, and *Cibotium schiedii*.

In the present paper an attempt has been made to study the range in structure of the antheridium of this family, and to ascertain if there is any variation in type which may be correlated with the divisions of the family. In the papers mentioned the antheridia of less than ten species have been described, belonging chiefly to the Cyathea. It should be noted that *Cyathea*, which is regarded as the most advanced genus of the Cyathea, has received most of the attention. It has been found that there is in every species a certain amount of variation from the type given as characteristic of the family. This seems to be related to the nutrition of the thallus, ordinarily, since the antheridium varies with the age and type of the thallus, that is, whether a normal heart-shaped thallus, a lateral shoot, or a filamentous ameristic prothallium. The present work indicates that in addition to these variations within any given species, there are other tendencies which seem to have some genetic significance.

The antheridia form ordinarily on the ventral surface; they may develop on the dorsal surface but are not marginal in the case of

typical heart-shaped prothallia; on filamentous ameristic prothallia they may be lateral or terminal (fig. 16). Fig. 65 shows an antheridium initial of *A. cooperi* on the anterior side of one of the wing cells. The type of antheridium which may be regarded as characteristic of the family was described by BAUKE with great care and completeness. The wall consists of five cells: a basal cell which is usually wedge-shaped; a lower ring (funnel) cell which is attached longitudinally to the antheridial wall by a connecting or binding wall (BAUKE's "Zwischenmembrane"); an upper ring cell; a crescent-shaped and an elliptical opercular cell formed by the division of the

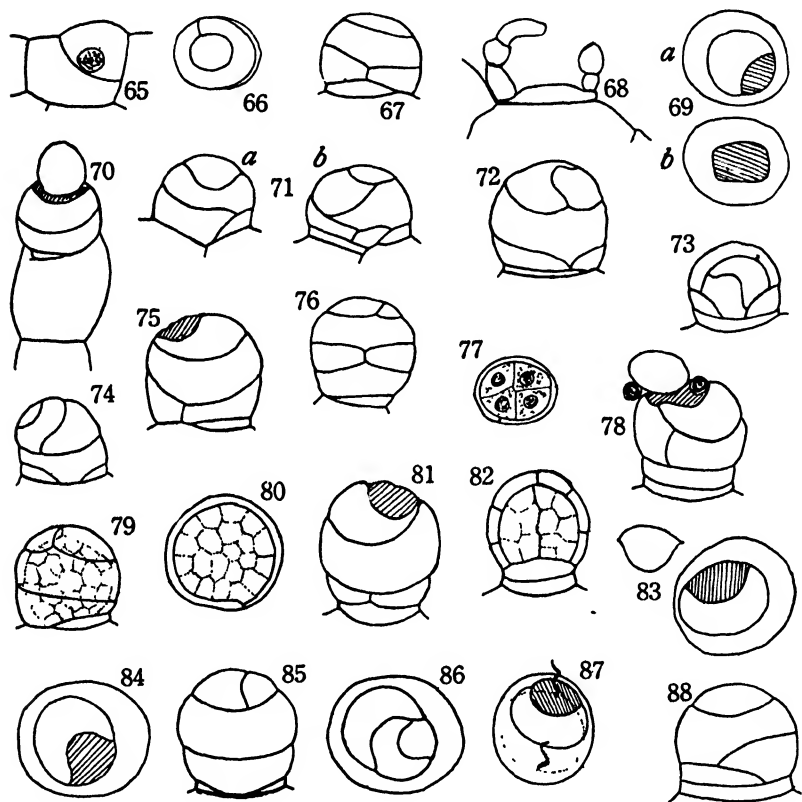


FIGS. 56-64.—*Lophosoria quadripinata*, $\times 300$.

primary cover cell. In general the antheridia on well developed prothallia of *Alsophila*, *Hemitelia*, and *Cyathea* correspond to this type (figs. 67, 72, 75, 81, 85); the antheridium of *Lophosoria* usually suggests a more primitive type (figs. 56, 58, 64), but the characteristic antheridium of the Cyatheaes is also found (fig. 57).

In typical antheridia the stalk cell is wedge-shaped and relatively thin, extending ordinarily from half to three-fourths across the base (figs. 66, 67, 75, 79) or even farther (fig. 85), but seldom completely across. In rare cases it divides vertically. In small antheridia, especially those on filamentous prothallia, the stalk cell may be absent. Sometimes there are two stalk cells (fig. 82). In stained preparations the stalk cell is strongly marked by the modification of its walls, which are often somewhat thicker than the other walls and

are usually heavily cutinized, so that the basal cell does not absorb water and swell as readily as do the other cells of the antheridium. The pronounced cutinization usually extends to the lower part of



FIGS. 65-88.—Figs. 65-72, *Alsophila cooperi*; figs. 73, 74, *A. armata*; figs. 75-77, *A. atrovirens*; figs. 78, 79, *A. excelsa*; fig. 80, *A. aspera*; fig. 81, *C. muricata*; fig. 82, *C. arborea*; fig. 83, *C. medullaris*; fig. 84, *C. dealbata*; figs. 85-87, *Hemitelia horrida*; fig. 88, *H. smithii*; all $\times 300$.

the funnel cell and may extend farther. The presence of the stalk cell is one of the characteristics which distinguishes the antheridium of the Cyatheae from that of the Polypodiaceae, and associates it with that of more primitive families.

The structure of the lower ring cell is another character which differentiates the Cyatheae from the Polypodiaceae, since the length-

wise attachment of the ring cell is not found in the polypods. Fig. 66 shows a young antheridium viewed from above in which there is a basal cell extending somewhat more than half across the base, the lower ring cell with its binding wall, and the more or less cylindrical cell in the center from which the dome cell is later cut off. Figs. 77 and 80 show the lower ring cell in cross-section. It sometimes happens that the two ends of this cell do not come together but connect with the antheridial wall at a noticeable distance apart (fig. 73). As BAUKE pointed out, the presence of the binding wall checks growth of the outer wall of the antheridium in the region of attachment, making the cell (and antheridium) asymmetrical (figs. 67, 68, 72, 79, 81). The second ring cell is formed from the dome cell and is more symmetrical. In some cases a binding wall is seen in this cell (fig. 78). It is possible that in such cases the second ring cell is formed in the same way as the first. BAUKE, however, thought it more probable that the wall was a secondary formation. Sometimes three ring cells are formed (fig. 76), making the antheridial wall a 6-celled structure.

The primary cap cell usually divides once (figs. 68, 69*a*, 75, 76, 78, 79, 81–85), but in large antheridia it may divide twice (fig. 86). In small antheridia it may remain undivided and is then identical with the polypod cap cell (figs. 69*b*, 70). The primary cover cell is formed by a circular wall which may be near the center of the dome cell, making a relatively symmetrical antheridium (figs. 76, 79, 85); or it may form to one side, making a distinctly asymmetrical antheridium (figs. 78, 87). Occasionally, especially in *Cyathea*, the walls of the opercular cell are modified and highly refractive (fig. 82). The opercular cell is thrown off at dehiscence (figs. 70, 78, 83) and, unlike the delicate opercular cell of the Polypodiaceae, may persist intact for many hours.

There are many variations from the typical antheridium. Reduction forms are frequent on filamentous ameristic prothallia. Additional cells may be present. In the antheridia shown in figs. 76, 82, and 86 the additional cell present may be regarded merely as a duplication of the lower ring cell, the basal cell, and the opercular cell respectively; but in such antheridia as those shown in figs. 71 (*a* and *b* are two views of the same antheridium), 73, 74, and 88 the

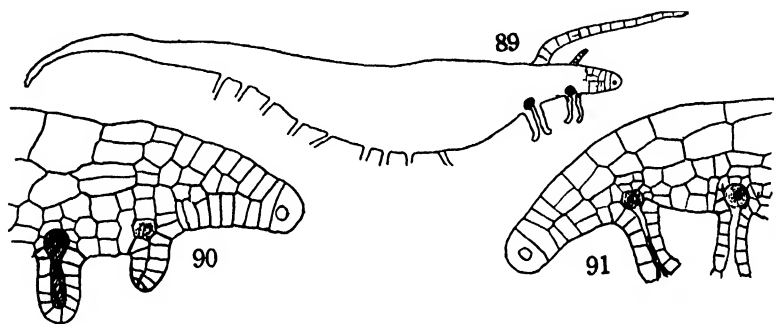
usual terminology does not fit. This type of irregularity is suggestive of the antheridia of primitive ferns.

The antheridia of *Lophosoria* stand somewhat apart from those of the other members of the Cyathea under investigation. They are somewhat larger on the average, and are more irregular in aspect. The number of sperms in cross-section ranges from eighteen to twenty-eight, averaging twenty-three or twenty-four, while in *Alsophila* and *Hemitelia* the number ranges from ten to twenty-five but is usually between fifteen and twenty. In *Cyathea* it is apt to be somewhat smaller. The larger size of the antheridia cannot be related to large prothallia, since the prothallia were rather undersized. Probably most of the antheridia in *Lophosoria* have a 5-celled wall, but the antheridium is usually asymmetrical and has the effect of being formed by a series of wedge-shaped cells. They are suggestive of the antheridia of *Gleichenia* or *Osmunda*, rather than of the Polypodiaceae. Fig. 59 shows a case of an antheridium which has proliferated and given rise to a second small antheridium. On one prothallium an imbedded antheridium was found (fig. 63). Since imbedded antheridia have been found to occur in the Polypodiaceae, as reported by BLACK (3) and FERGUSON (9), it is surprising that this is the only case found in this family.

The antheridia of *Cyathea* seem to approach those of the polypods rather more closely than do those of *Alsophila* and *Hemitelia*. They are inclined to be somewhat smaller and rather more symmetrical. BOWER (5) has emphasized the relation between the size of sporangia and antheridia. *C. dealbata* is of interest because of the small spore output. The range in size of antheridia in any species is so great that it is difficult to be positive about slight differences. The antheridium in *C. dealbata* is not large, but it can hardly be said that it is obviously smaller than that of other species of *Cyathea*; the one shown in fig. 84 represents probably the maximum size, and that is not the case for the figures of the antheridia of the other species. The difference between the antheridia of *L. quadripinnata* and those of *C. dealbata* both in size and symmetry is striking, but it is not possible to speak so positively about the various species of *Cyathea* in which differences are slight if they exist at all.

ARCHEGONIUM.—As is the case in all typically heart-shaped pro-

thallia, the appearance of archegonia depends upon the development of the cushion. In the Cyathea archegonia do not appear as early in the development of the prothallium as in the Polypodiaceae, that is to say, the prothallium is later in arriving at maturity. In polypod prothallia mature archegonia may be found on prothallia in which the adjacent tissue is only two cells thick and there is no vegetative part any thicker. No cases of this were seen in the Cyathea. In a few cases mature archegonia were found on a thallus in which the adjacent tissue was only three cells thick, but usually it was at least four cells thick. The prothallia shown in figs. 89-91



FIGS. 89-91.—*Alsophila cooperi*: fig. 89, $\times 45$; fig. 91, $\times 105$; fig. 90, *A. excelsa*, $\times 105$.

are relatively young. Figs. 89 and 91 show sections of prothallia made eight weeks after the spores were planted; that shown in fig. 89 had ten mature archegonia and was six cells thick in the heaviest part, the other had only six archegonia. Fig. 90 shows a prothallium which had twelve archegonia.

The archegonium initial (fig. 93) appears near the apical region, usually 3-5 cells from the apex. The first division in the initial cuts off the primary neck cell (figs. 92a, 94) and the next forms the basal cell (figs. 94-96). Fig. 96 shows a "row of three" in *C. medullaris* formed from a wedge-shaped initial; this is a rare type in the Cyathea. BAUKE (1) states that in the Polypodiaceae two basal cells are formed occasionally, but that in certain species of the Cyatheaaceae it is the rule. That cannot be said to be the case in any species included in this investigation. While the formation of two basal

cells is of frequent occurrence (fig. 98), and even three may be formed (fig. 104), the archegonium with one basal cell is the usual type and that with two the exception. In the species of *Hemitelia* under investigation cases of two basal cells were more frequent than in the other genera; but BAUKE states that *H. spectabilis* did not produce two basal cells, while *C. medullaris* and *A. australis* did regularly. In the archegonium of *H. horrida* (fig. 102) the two basal cells were cut off obliquely. In *Lophosoria* cases of two basal cells are rare. It may be that the tendency to form two basal cells is related to the tendency of the Cyatheaes to develop a thicker thallus than that of the polypods; the individual cell, as well as the prothal-

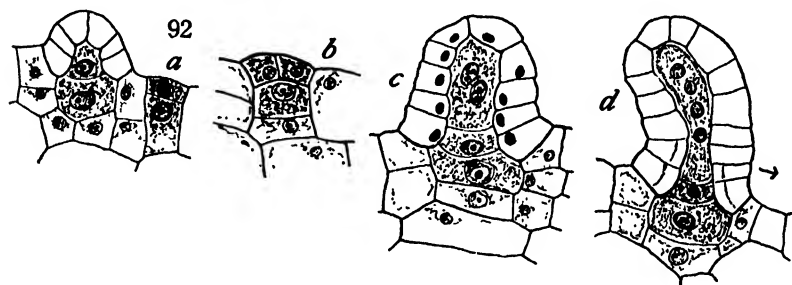
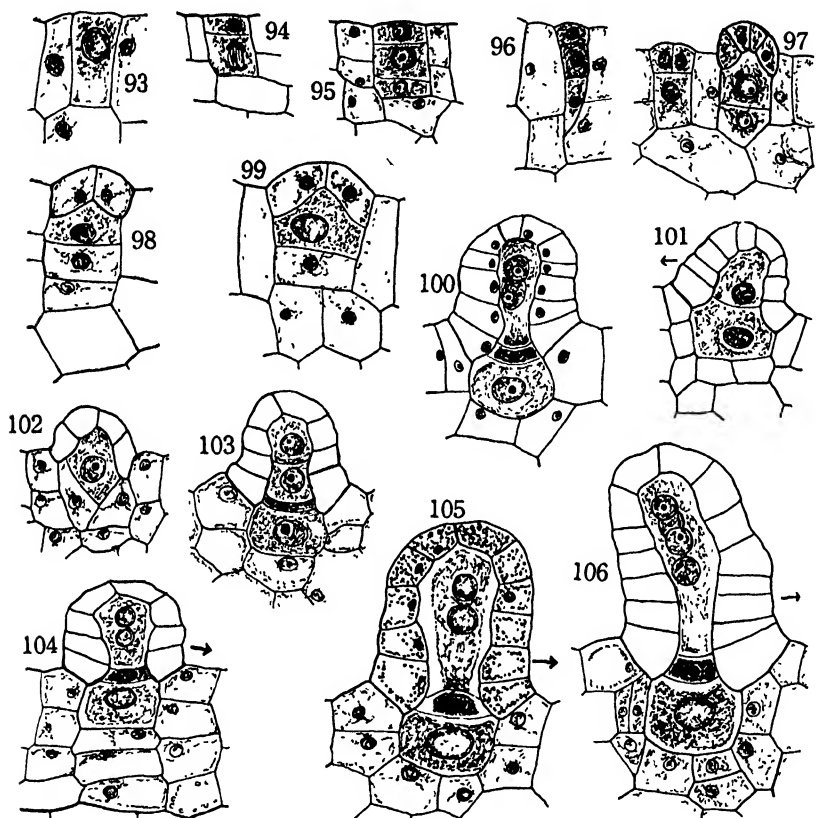


FIG. 92.—a-d, *Lophosoria quadripinnata*, $\times 300$

lium as a whole, not maturing so rapidly. The origin of the second basal cell was not determined as no mitotic figures were seen. The production of two oblique cells, which was seen several times, indicates that both may be formed from the central cell. In several cases, however, the basal cell divided vertically at an early stage (figs. 92a, 101), and this suggests that the second basal cell is formed by the division of the primary basal cell. ROGERS (22), in discussing the question of basal cells in *Lygodium*, suggests that in many cases the so-called second basal cell is not a true basal cell in the sense that it is a product of the archegonium initial. It is certainly true that many cases were seen in which a cell was present which might have been derived from the thallus beneath the archegonium initial and yet looked like a second basal cell, but in other cases the second basal cell was undoubtedly a product of the archegonium initial.

The arching of the neck usually begins with the division of the

primary neck cell into the four cells which form the initials for the four rows of neck cells; the central cell then pushes up between the neck cells (figs. 92*b*, 98, 99). Figs. 92*a*, 97, and 101 illustrate the



FIGS. 93-106.—Figs. 93, 94, 97, 98, 102, 104, 105, *Hemitelia horrida*, $\times 300$; figs. 95, 101, *Alsophila excelsa*; figs. 96, 99, *Cyathea medullaris*; fig. 100, *A. cooperi*; fig. 103, *H. smithii*; fig. 106, *C. dealbata*; $\times 300$.

next stages in the development of the archegonium, the further divisions of the neck cells and the division of the central cell to form the primary neck and the primary ventral cell. Figs. 92*c*, 100, 104, and 105 show the mature archegonium in which has occurred the division of the primary ventral cell to form the egg and ventral canal cell, and the division of the primary neck canal-nucleus. The

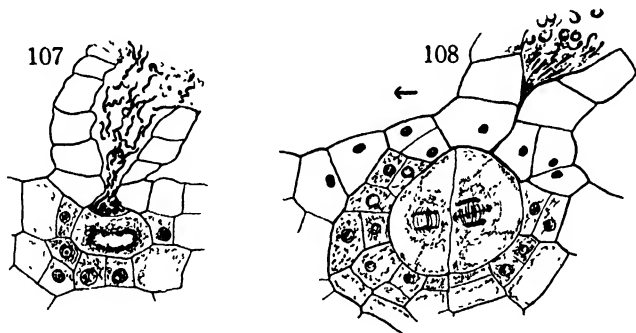
neck canal nucleus ordinarily divides once, giving the mature archegonium two neck canal nuclei, but the formation of four neck canal nuclei is of rather frequent occurrence (figs. 92d, 106), and cases were found in all the species studied. TWISS (28) has reported it for *Lygodium circinatum*, ROGERS (22) for *L. palmatum*, and PFEIFFER (21) for *Pteris longifolia*. Only one archegonium was found in which there was a wall separating the neck canal nuclei; this was in *H. smithii* (fig. 103).

In the character of the mature archegonium (the length and straightness of the neck) the Cyatheaes ally themselves with the more primitive families, the Osmundaceae and the Gleicheniaceae, rather than with the Polypodiaceae. The necks are noticeably longer and straighter than those of polypods, with the anterior side having only one more cell than the posterior and with very little elongation of the anterior cells. The curve, when present, is usually away from the notch but may be toward the side, or even toward the notch. The position of the apical region is indicated in the illustrations by the arrow. The longer side of the archegonium is usually six or seven cells long, occasionally eight, rarely five; while in the polypods it is usually four or five, less frequently six. Archegonia which have four neck canal nuclei are usually somewhat longer than the typical archegonium, and are more likely to be curved.

In mature and even in young archegonia there is a striking difference between the contents and walls of the neck cells and those of the venter or of the other cells of the thallus. This differentiation may appear just after the first division of the primary neck cell, or it may not be evident until several divisions have taken place. In fresh material the neck cells are pale and have coarsely granular contents; in stained preparations the contents may show as conspicuously stained granules, or the contents may stain rather uniformly, suggesting a mucilaginous character. The reactions of the neck cells suggest a heavy cutinization. The reaction to killing and fixing agents is unlike that of the venter of the archegonium or the vegetative part of the thallus; it is more difficult to get effective penetration in the neck cells. This is probably the reason why formalin alcohol usually gave better results as a killing agent than chromo-acetic acid. The peculiar character of the walls is most marked in slightly faded preparations which have been stained with

Flemming's triple stain, as the neck cells retain the stain much better than the others. The archegonium in such cases may have the appearance of a cap set in the thallus. This modification of the neck is not characteristic of the Polypodiaceae, but is found in such primitive families as the Osmundaceae.

As the archegonium matures there may occur divisions in the cells surrounding the egg (figs. 106, 107); secondary divisions may also occur in the neck cells (fig. 92*d*). If water is present at this time the archegonium opens and pours out the more or less mucilaginous contents of the canal. The reaction of the sperms in the region of the archegonium is very pronounced; instead of swimming



FIGS. 107, 108.—Fig. 107, *A. cooperi*, $\times 300$; fig. 108, *Cyathea dealbata*, $\times 300$.

irregularly they dart toward the mouth of the archegonium and enter, often in great numbers (fig. 107), straightening and elongating as they penetrate the mucilaginous substance. For some time after fertilization the remains of sperms may be seen in the neck (fig. 108).

The first division of the fertilized egg is parallel to or in the general direction of the axis of the archegonium.

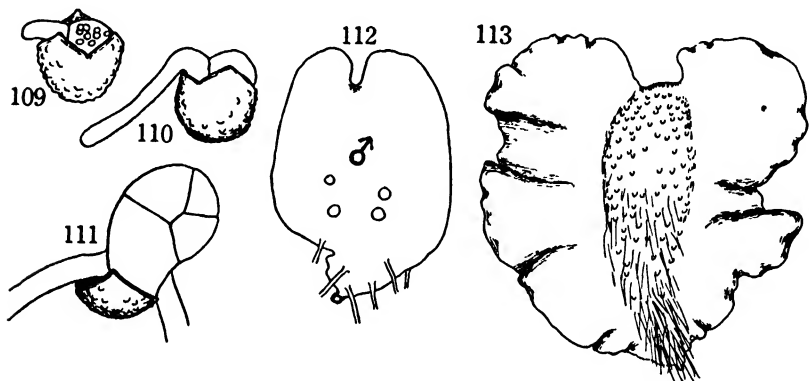
In an earlier paper (27) an account was given of the occurrence of apogamy in the Cyatheaceae. Since then apogamous growths have been found in *Lophosoria quadripinnata*, *A. excelsa*, *A. armata*, *H. parvula*, *C. medullaris*, and *C. dealbata*.

Thyrsopterideae

The spores of the rare endemic fern of Juan Fernandez, *Thyrsopteris elegans*, were obtained from the Royal Botanic Gardens, Kew, through the kindness of Mr. L. M. BOODLE.

VEGETATIVE STRUCTURE

The spores of *T. elegans* are of the tetrahedral type, dark brown in color, with a thick coat which has abundant irregular warty or spiny processes. They range in diameter from 210 to 275 μ , averaging 230–250 μ . Germination, with spores 2–3 weeks old, occurred in 4–7 days. The difference in rate seems to be related chiefly to temperature. Germination was obtained in spores nine months old, but



FIGS. 109–113.—*Thyrsopteris elegans*: figs. 109–111, $\times 165$; fig. 112, $\times 30$; fig. 113, $\times 7$.

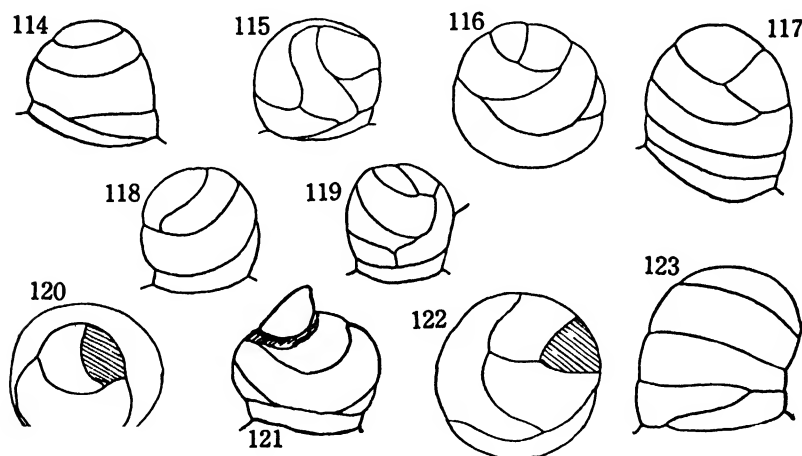
the percentage of viable spores was much lower than in fresh spores. The early stages are shown in figs. 109–111. Germination is similar to that found in the Cyatheae; there is usually no filament formed, even when spores are much crowded, but a plate is formed immediately by longitudinal or oblique divisions in the second or third cell. The prothallium elongates after the plate has formed but does not broaden rapidly at this stage (fig. 112). The prothallium when it reaches maturity (archegonial production) is like that of the Cyatheae in being relatively long and having relatively less wing than is usually found in the prothallia of the Polypodiaceae. Mature prothallia may attain considerable size, some reaching a length of 2 cm. The rib is rather wide but not especially thick, ordinarily not exceeding seven cells in thickness. The development of the rib is such that the wings are often lifted up so that the prothallium is U-shaped in cross-section. The wings may make a sufficiently

luxuriant growth to give an old prothallium a more or less ruffled appearance. Forking occurred not infrequently in prothallia over a year old, the branches being rather narrow but thick. One forking prothallium 2 cm. long had two long branches 3-5 mm. in width.

No hairs, either multicellular or of the polypod type, were found on the prothallia of *Thyrsopteris*, even on old vigorous prothallia which had 100-200 archegonia.

REPRODUCTIVE STRUCTURES

ANTHERIDIUM.—The antheridia of *Thyrsopteris* are distinguished by their size and asymmetrical aspect (figs. 114-123). They can

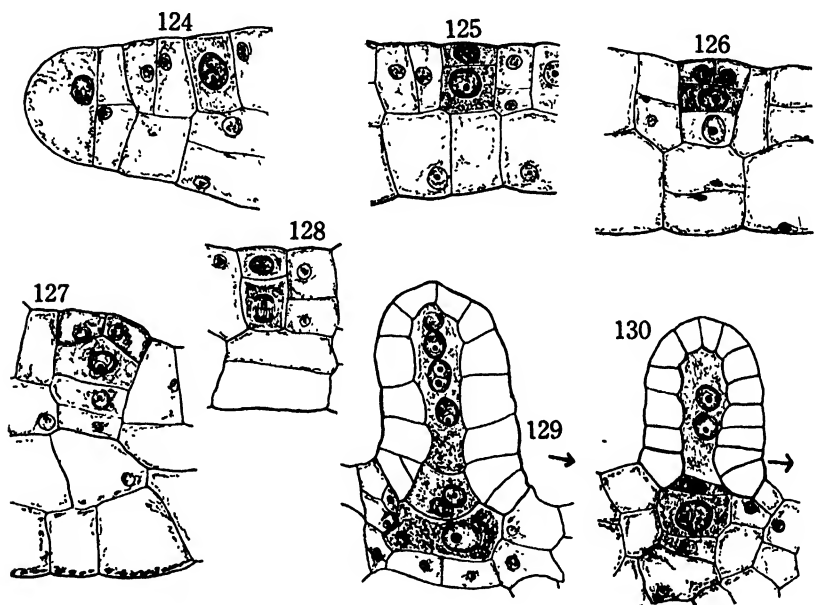


FIGS. 114-123.—*Thyrsopteris elegans*, $\times 300$.

hardly be described in terms of the antheridia of the Cyathea or of the Polypodiaceae. They are similar to some of the largest and most irregular of the antheridia of *Lophosoria*, or to those of the Osmundaceae. A basal cell can usually be distinguished, but there may be two or even three basal cells. The opercular cell, thrown off at maturity, is perfectly definite (fig. 121). In the majority of antheridia, however, it is hardly possible to designate cells as funnel, ring, or primary cover cells. Even when the antheridium apparently consists of five cells of the cyatheoid type it may be large and asymmetrical, and it is difficult to be sure of the number of cells.

This type, when it exists, is doubtless to be looked upon as a reduction form rather than as the characteristic type. The antheridia apparently are formed by a series of wedge-shaped cells. While many antheridia appear to have a primary cap cell which divides twice (fig. 120), there are many which cannot be interpreted in this way (fig. 122).

ARCHEGONIUM.—The archegonium of *Thyrsopteris* appears at about the same stage of development of the thallus as in the Cya-



FIGS. 124-130.—*Thyrsopteris elegans*, $\times 300$.

theae. Figs. 124-128 show sections of the thallus in which the relation of the initial and the young archegonium to the thickness of the thallus can be noted. There is usually only one basal cell (fig. 126) but two are formed occasionally (fig. 127). The neck of the archegonium is longer, as a rule, than those of the Cyathea; it may be straight but is usually more or less recurved or curved to the side. The same type of modification of the contents and walls of the neck cells occurs as has been described for the Cyathea. Archegonia with four neck canal cells are not infrequent; in the archegonium

shown in fig. 129 the nucleus of the ventral canal cell had also divided. The position of the wall in the first division of the embryo was not observed.

Thyrsopteris shows a strong tendency to form apogamous growths. This probably accounts for the difficulty experienced in raising sporelings of this species. Mr. BOODLE wrote to me several years



FIG. 131.—*Thyrsopteris elegans*, sporeling, $\times 1$ (photograph by A. S. KINNEY).

ago that they had been unsuccessful at Kew. Although working with *Thyrsopteris* for several years, I have succeeded in raising only one sporeling which has progressed far enough to give hopes of raising it to maturity (fig. 131). The apogamous plants developed vascular structures and the characteristic leaves but did not produce stem or root.

Dicksonieae

The following list gives the eleven species of the Dicksonieae which were investigated and the length of time each was kept in culture.

*Culcita macrocarpa*² Presl. = *Balantium culcita* (L'Hérit.) Klf.
2 and a half years

Cibotium barometz (L.) J. Sm. 5 years

Cibotium chamiissoi Klf. 2 and a half years

Cibotium glaucum (Sm.) Hk. & Arn. 7 months

Cibotium menziesii Hk. 2 and a half years

Cibotium regale Linden 1 year

Cibotium schiedii Schlecht. & Cham. 7 months, 8 months, 1 year

Dicksonia antarctica Lab. 6 months, 8 months, 2 years

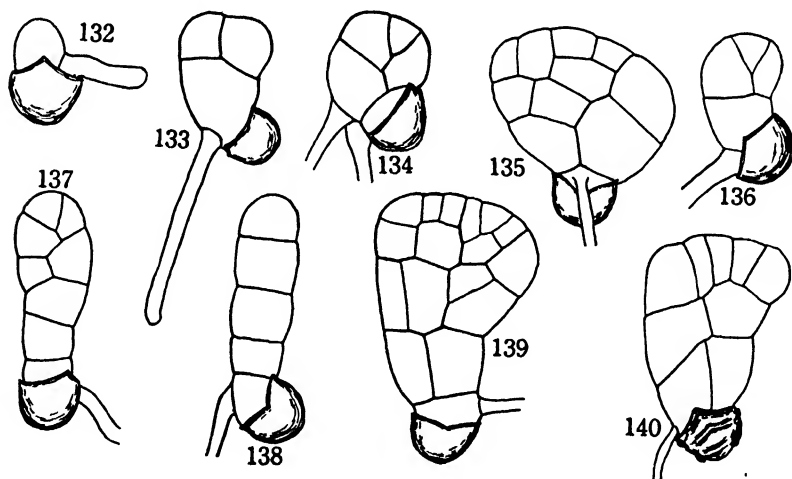
Dicksonia fibrosa Col. 15 months

Dicksonia squarrosa (Forst.) Sw. 8 months

Dicksonia youngiae C. Moore 9 months

VEGETATIVE STRUCTURE

Germination and the early stages of the prothallium in this group are shown in figs. 132-140. It will be seen that the same type pre-

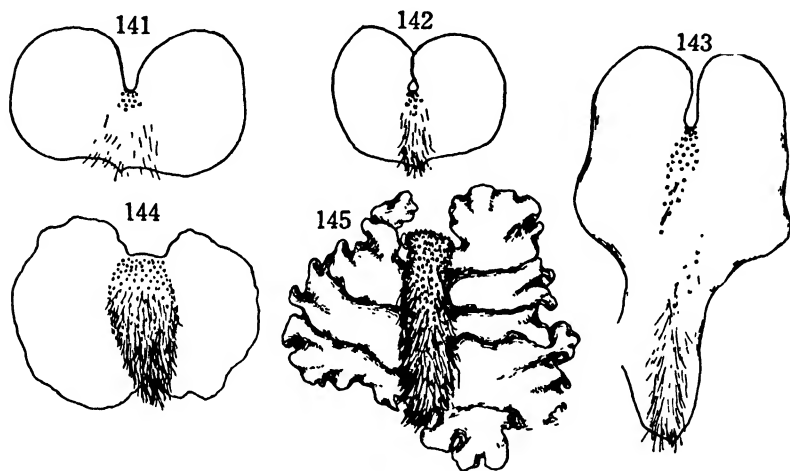


FIGS. 132-140.—Fig. 132, *Cibotium schiedii*, $\times 165$; figs. 133-135, *Dicksonia antarctica*; figs. 133-135, $\times 165$; fig. 134, $\times 185$; fig. 136, *D. fibrosa*, $\times 165$; figs. 137-139, *C. regale*, $\times 165$; fig. 140, *C. barometz*, $\times 150$.

vails here that was found in the *Cyatheae* and *Thyrsopteris*. The young prothallium passes quickly into the plate stage, leaving at

² In the name of this species I have followed MAXON (19) instead of the nomenclature of CHRISTENSEN.

the base in most cases only one cell in which there is no longitudinal division. The tendency to form a filament is very slight in most species, even in crowded cultures. Fig. 140 shows a prothallium of *Cibotium barometz* taken from a clump of 25-30 young prothallia; in the whole clump there were only three prothallia which had more than one cell at the base without longitudinal divisions. The tendency to form a filament is somewhat stronger in *C. regale*, as prothallia of the type shown in figs. 137 and 138 are not uncommon,



FIGS. 141-145.—Fig. 141, *Dicksonia fibrosa*, $\times 5$; figs. 142, 145, *Cibotium schiedii*; fig. 142, $\times 5$; fig. 145, $\times 2.5$; fig. 143, *C. menziesii*, $\times 6$; fig. 144, *Culcita macrocarpa*, $\times 2.5$.

but most of the cells in such filaments divide longitudinally or obliquely after a time (fig. 139).

The prothallia of *Cibotium* and *Dicksonia* when 2-3 months old as a rule are more like those of the polypods than those of the Cyathea or *Thyrsopteris* (figs. 141, 142); the prothallia are distinctly broad and short with considerable wing development. If fertilization does not take place and the prothallia are kept under favorable conditions the rib develops more heavily, and the prothallia become more massive than any of the Cyathea which were studied. If the cultures are somewhat crowded the prothallia elongate, but when given an opportunity they broaden rather rapidly

(fig. 143). The rib may become heavy and cause the wings to be lifted; or the wings may grow luxuriantly, producing the curly ap-

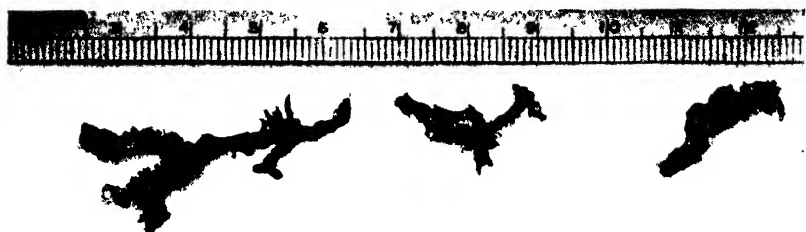
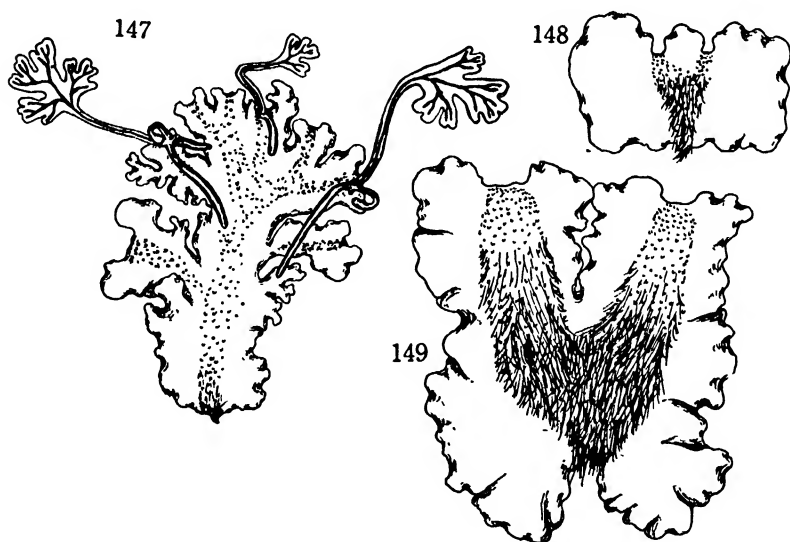


FIG. 146.—*Cibotium barometz*; prothallia four years old, $\times 1.25$ (photograph by A. S. KINNEY).

pearance shown by *Cibotium schiedii* (fig. 145). Forking of the meristem with the production of one or more archegonium-bearing



FIGS. 147-149.—Fig. 147, *Cibotium barometz*, $\times 2.5$; figs. 148, 149, *Dicksonia antarctica*, $\times 2.5$.

branches occurred in *Culcita macrocarpa*, *Cibotium barometz*, *C. chamissoi*, *C. schiedii*, *Dicksonia antarctica*, and *D. fibrosa*. The

prothallia of *C. barometz*, although not exceptionally thick or broad, elongated rapidly and forked freely. The prothallia shown in fig. 146 were from a 4-year old culture. As shown by fig. 147, each branch of the prothallium may act as a unit in the production of sporelings. Forking of the meristem occurred at an earlier stage in *D. antarctica*; the branches were heavier, as a rule, and did not elongate to the same extent. The cushion of such a prothallium as that shown in fig. 149 may become 12-14 cells thick.

No hairs of the type characteristic of the Cyatheaes were found on the prothallia of any species of the Dicksonieae included in this investigation. No hairs of any type were found except in the case of a single prothallium of *C. barometz* which bore the structure shown in fig. 150a, which is apparently a cylindrical process which may be looked upon as apogamous. These hairs are like those on the young petiole of *C. barometz*, and are quite unlike the hairs of the Cyatheaes either in form or origin. This species has been found to produce apogamous growths, and while this process is not definitely sporophytic it shows a wide departure from any gametophytic structure.

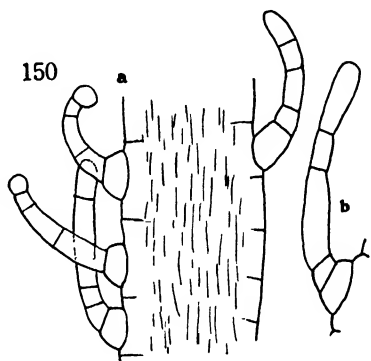


FIG. 150.—a, b, *Cibotium barometz*,
×105.

BAUKE (1) states that in none of the prothallia of the Polypodiaceae which he studied did he find bristle-like hairs, but that he found them in all of the Cyatheaes. This would imply their presence in *D. antarctica* and *C. schiedii*, but these two species are among the forms which he expressly states were less fully studied than *Cyathea medullaris*, *H. spectabilis*, and *A. australis*, chiefly owing to lack of material. HEIM (13) reported hairs for *D. antarctica* (*Balanium antarcticum*), and described them as occurring not only on the dorsal and ventral surfaces but also on the margin, but he gave no figures showing the type of hair. I am unable to agree with the accounts of BAUKE and HEIM with regard to the presence of hairs on *D. antarctica* and *C. schiedii*. Many prothallia of both species have

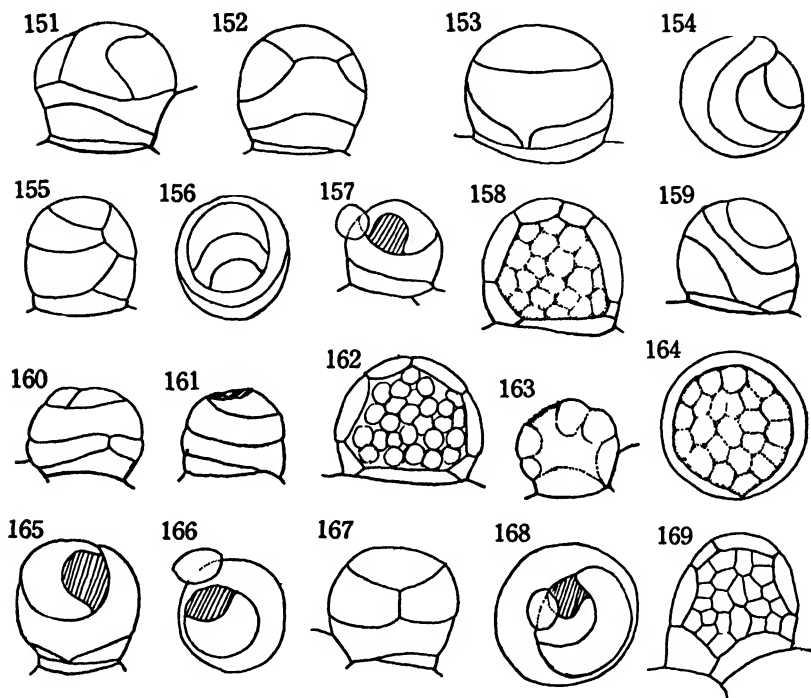
been examined but not a single hair either unicellular or multicellular has been seen. In the case of *D. antarctica* three sets of cultures were carried, two from spores obtained from Fairmount Park, Philadelphia, and one from spores from Kew. The three sets of cultures were similar in habit and range of type. The Kew cultures were kept for over two years. The prothallia were vigorous and grew to a large size for that species (15 mm. in length), many cases of branching occurred, archegonia were produced in great numbers (300-400 on a prothallium). This indicates sufficient maturity for the appearance of much belated hairs but none appeared.

REPRODUCTIVE STRUCTURES

The prothallia of the Dicksonieae are monoecious with a rather more pronounced tendency toward dioecism than was seen in the Cyatheaes. Many prothallia passed at once to the production of archegonia without apparently any antheridial period. If the prothallium went through an antheridial period the production of antheridia ceased as soon as archegonium production was initiated. Antheridia were usually produced most abundantly on special male prothallia. In most cases these were flat plates with a weak meristem which might persist for many months. In some cases ameristic male prothallia were formed but they were usually more or less elongated plates, rarely filaments. The formation of ameristic filaments is much less frequent in the Dicksonieae than in the Cyatheaes.

ANTHERIDIUM.—The antheridia of the eleven species of the Dicksonieae which were studied are, with the exception of those of *Culcita macrocarpa*, of the same general type as those of *Alsophila*, *Hemitelia*, and *Cyathea*, but are usually somewhat larger (figs. 158, 162-164, 165). They may show from fifteen to thirty-eight sperms in median section, but the usual number is between seventeen and twenty-five (figs. 158, 162, 164). The tendency for the primary cap cell to divide twice (figs. 156, 168) is somewhat stronger than in the Cyatheaes, and there are fewer cases of undivided cap cells. The antheridium of *Culcita macrocarpa* is interesting because of its suggested primitiveness. While not larger than others in this subfamily, it is decidedly more irregular and asymmetrical (figs. 151-155). The number of cells in the wall is regularly five but frequently

six. The walls of the cell are oblique and often suggest wedge-shaped cells formed from an apical cell rather than basal, funnel, and ring cells. In many cases the antheridium when viewed from the top appears to be crossed by walls more or less parallel (fig. 154), and

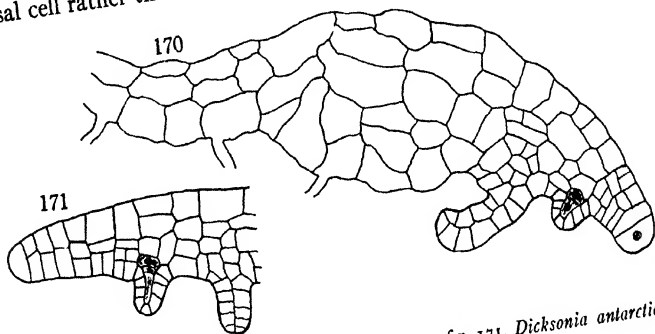


FIGS. 151-169.—Figs. 151-155, *Culcita macrocarpa*; figs. 156, 157, *Dicksonia antarctica*; fig. 158, *D. fibrosa*; fig. 159, *D. squarrosa*; figs. 160-163, *Cibotium chamissoi*; fig. 164, *C. regale*; figs. 165, 166, *C. barometz*; fig. 167, *C. schiedii*; figs. 168, 169, *C. menziesii*; $\times 300$.

there is nothing to suggest a primary cover cell such as is seen in figs. 156, 166, and 168. In the case of the antheridium of *Culcita macrocarpa* it is somewhat difficult to use the terminology which applies to the typical antheridium of *Dicksonia* and *Cibotium*.

ARCHEGONIUM.—In the Dicksonieae archegonial formation begins when the thallus is at the same stage as that noted in the Cyathea and *Thyrsopteris*, or even later (figs. 170-172). Figs. 170 and 171 show sections of small prothallia; the one in fig. 170 had six arche-

gonia and that in fig. 171 had ten. Fig. 172 is taken from a prothallium of *Cibotium barometz* which had no mature archegonia and one but slightly older than the one shown; the thallus had already attained a thickness of four cells. Cases of two superimposed basal cells are much less frequent than in the Cyatheaes, but the rather frequent occurrence of a vertical division in the basal cell of *Culcita macrocarpa* and *Cibotium barometz* (figs. 174, 177), and its occasional occurrence in other species, suggest that the second basal cell in the superimposed type (fig. 178) is formed by a division of the primary basal cell rather than by a division of the central cell. The appear-

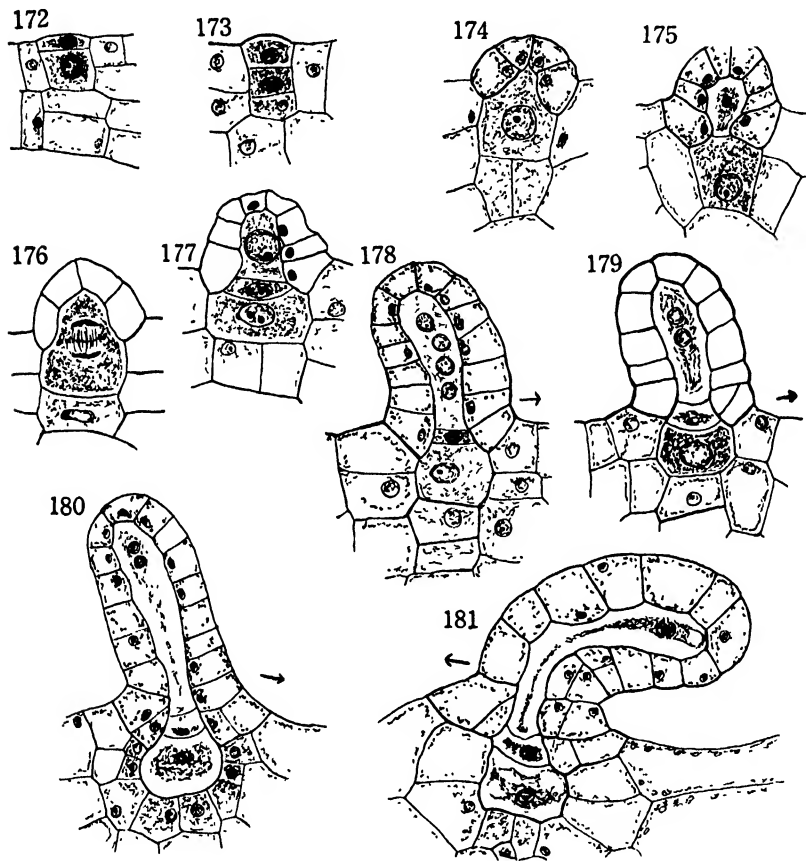


FIGS. 170, 171.—Fig. 170, *Cibotium schiedii*, $\times 105$; fig. 171, *Dicksonia antarctica*, $\times 105$.

ance of the contents of the cells does not indicate that this is a pre-mature appearance of the divisions leading to the formation of the nutritive jacket of the egg such as is shown in fig. 180. Fig. 175 shows a young archegonium which has no basal cell; this is the only case which was found.

The neck of the archegonium in the Dicksonieae is decidedly longer than in the Polypodiaceae and usually longer than in the Cyatheaes; it is most like that of *Thyrsopteris*. The neck is usually seven or eight cells long on the anterior side at maturity (figs. 178, 181), rarely six (fig. 179), and occasionally nine or even ten (fig. 180). The tendency to curve away from the notch is more pronounced than in the Cyatheaes, especially in *Cibotium* which on the whole has longer necks than *Dicksonia*. The same type of modification of contents and wall of the neck cells which was noted in the

Cyatheae and *Thyrsopteris* is found in the Dicksonieae. Archegonia with four neck canal nuclei (fig. 178) were found in all species included in this investigation.



FIGS. 172-181.—Fig. 172, *Cibotium barometz*, $\times 300$; figs. 173, 179, 180, *C. regale*; figs. 173, 179, $\times 300$; fig. 180, $\times 245$; fig. 174, *Culcita macrocarpa*, $\times 300$; figs. 175, 176, 178, *Dicksonia antarctica*, $\times 300$; fig. 177, *D. squarrosa*; fig. 181, *Cibotium schiedii*, $\times 300$.

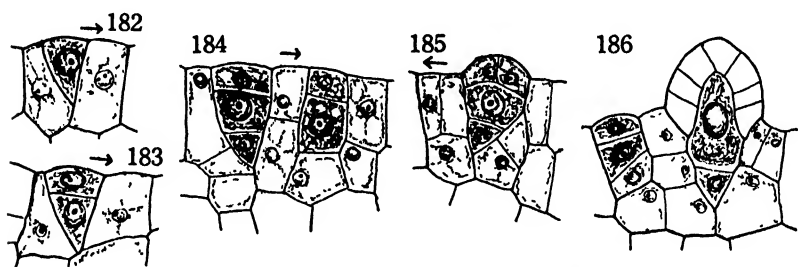
The formation of archegonia from wedge-shaped initials is found more frequently in this group than in the Cyatheae (figs. 182-186). It was found most frequently in *Cibotium barometz* but it occurs not infrequently in *C. regale* and other species in this group.

The first wall of the embryo is approximately in a lengthwise plane of the archegonium.

In addition to the case of apogamous growths reported for *D. squarrosa* in an earlier paper (27), apogamy was found in *C. barometz* and *C. schiedii*.

Discussion

The points on which this investigation should shed light are whether or not the group is a natural one, and the connections both downward and upward shown by the group as a whole or by its members. HORVAT (14) has given a discussion of the significance of the gametophyte of the ferns in phylogeny with full references to the literature on the subject. The lines of evidence presented by the gametophyte which have commended themselves most strongly



FIGS. 182-186.—Figs. 182, 183, 185, 186, *Cibotium barometz*; fig. 184, *C. regale*; $\times 300$.

to investigators are the structure of the antheridium and the character of the hairs, if any are present. Germination characters and the habit of the mature prothallium, owing to their variability, and archegonial characters, because of their uniformity, have been rated less highly.

In the structure and development of the antheridium there is some uniformity, except for *Lophosoria* and *Thyrsopteris*; *Culcita macrocarpa* may also be given as not conforming to the type. *Thyrsopteris*, which stands out most sharply as a primitive type in antheridial structure, is also set apart by its sporophytic structure. These three genera are obviously not closely related to one another; it will hardly be questioned that *Lophosoria* is more closely related to *Alsophila* than it is to *Thyrsopteris* or *Culcita*, and that *Culcita* is related to *Cibotium* and *Dicksonia* rather than to *Lophosoria* and *Thyrsopteris*. The antheridia of these three forms are similar in their

irregularity and lack of symmetry; those of *Lophosoria* and *Thyrsopteris* also stand apart in the matter of size. They agree in being suggestive of a primitive type such as that of *Osmunda* or *Todea*, rather than that of the Polypodiaceae. The similarity in type of the antheridium of *Alsophila*, *Hemitelia*, and *Cyathea* to those of *Cibotium* and *Dicksonia* can be explained as due to a reduction from the type of the Osmundaceae which has reached the same stage in both lines. All fern antheridia may be derived from a type formed by an apical cell, as suggested by STEPHENSON (25) and shown diagrammatically by GOEBEL (11). In the process of reduction and simplification the same type may be reached in several lines. The antheridium of the three subfamilies may be derived readily from the *Osmunda* type. Those of *Gleichenia* are intermediate or at the *Thyrsopteris* stage; in the Hymenophyllaceae and the Schizaeaceae, apparently, reduction and simplification have gone further. So far as the evidence from the antheridia is concerned there is nothing to indicate that the Cyathea, Thyrsopterideae, and the Dicksonieae have a common ancestor nearer than the Osmundaceae.

The multicellular hairs found on the prothallia of *Lophosoria*, *Alsophila*, *Hemitelia*, and *Cyathea* place the group of the Cyathea sharply in contrast to the Dicksonieae and *Thyrsopteris*. The type of hair is peculiar in its origin, structure, and distribution. It can hardly be related to any other type except those found on species of *Gleichenia* and perhaps those of *Loxsonia*. In origin and distribution the hairs of the Cyathea are like those of *Gleichenia*. The hairs of *Gleichenia* differ in being only 2-3 cells long and ending usually in a somewhat swollen tannin-filled tip, while those of the Cyathea may be 20-30 cells long and 8-10 wide, never ending in a glandular tip. The similarity in mode of origin and distribution is certainly indicative of a genetic connection between the Gleicheniaceae and the Cyathea. The comparison which has frequently been made since the appearance of the work of SCHLUMBERGER (23) of the hairs of the Cyatheaceae to those of *Woodsia* and *Diacalpe* seems not to be significant; the difference in origin and distribution opposes rather than favors a genetic connection. The rare and belated appearance of hairs in *Lophosoria quadripinata* emphasizes the separation between *L. quadripinata* and *Alsophila*. The evi-

dence from the hairs is that the Cyatheae are rather closely related to the Gleicheniaceae and probably to *Loxsoma*, but that the connection with the Dicksonieae and *Thyrsopteris* is remote.

As for the question of germination, it is uncertain what value is to be placed on it owing to the plasticity of the prothallium at this stage. In general the prothallia of the Cyatheaceae are less plastic than those of the Polypodiaceae at this stage; the habit of the prothallium in the early stages sets them somewhat apart from the Polypodiaceae but does not definitely ally them with any lower forms. The type which is characteristic of the three subfamilies is distinctly different from that of the Osmundaceae; it has been found in *Gleichenia* spp. and in species of the Schizaeaceae. At present there is probably not enough known about germination types in the lower families and the degree of plasticity which exists to enable valid deductions to be made.

As to the mature prothallium, one character which is more primitive than the polypods is their later maturation, the later attainment of the antheridial and the archegonial stages. Perhaps the tendency of the apical region to fork and produce branching prothallia should be mentioned; and it might be noted that the polypods do not ordinarily develop so thick a thallus, at least in prothallia 8-12 weeks old, as is found in the Cyatheae, Dicksonieae, and *Thyrsopteris*. While in long-continued cultures, as MOTTIER (20) has shown, large forking prothallia may be produced by polypods (for example, *Matteuccia nodulosa*) as well as by more primitive ferns such as *Osmunda regalis*, forking is not initiated at as early a stage as in *Hemitelia* and *Dicksonia*. The tendency to produce filamentous ameristic male prothallia which bear antheridia at an early stage is very much more pronounced in the Polypodiaceae than in the Cyatheae, and it is stronger in the Cyatheae than in the Dicksonieae and *Thyrsopteris*. The early production of antheridia and archegonia increases the probability of early fertilization and sporophyte production. In pushing forward these processes the Polypodiaceae are definitely in advance of the Cyatheae, Dicksonieae, and *Thyrsopteris*, with the Cyatheae approaching them.

The evidence given by the gametophyte stage indicates that the family Cyatheaceae as given by CHRISTENSEN (7) and DIELS (8) is

not a natural group. It gives strong justification for the splitting of the family into two parts, and supports the position taken by BOWER that it should be divided, the main division following the line which he has established on the basis of the origin of the sporangia. It would be too much to say that the evidence from the gametophyte supports the further division of the cyatheoid line and the establishment of the family Protocyatheaceae, but it may be well to point out that the gametophytic characters of *Lophosoria quadripinnata* emphasize the inappropriateness of retaining it in the genus *Alsophila*, and present no difficulty in placing it in a separate family. The gametophyte of *Thyrsopteris elegans* has more in common with those of *Culcita*, *Cibotium*, and *Dicksonia* than with those of any other group, and so far as the evidence from the gametophyte is concerned there is no reason why it should not be included in the Dicksoniaceae.

Summary

1. Under favorable conditions the plate stage follows very quickly after germination; there is ordinarily no filament left at the base of the prothallium. The mature prothallium usually is longer and the cushion heavier than in the Polypodiaceae. Forking of the cushion of the prothallium occurs frequently in old cultures, and in certain species may occur in relatively young prothallia.

2. Multicellular hairs were found to develop more or less abundantly on mature prothallia of all species of *Alsophila*, *Hemitelia*, and *Cyathea*, and to occur rarely on *Lophosoria*. None were found on *Thyrsopteris*, *Culcita*, *Cibotium*, or *Dicksonia*. The multicellular hair arises from a special initial, a wedge-shaped cell cut from the anterior face of a superficial cell near the apical meristem. The hairs are found on both dorsal and ventral surfaces, on or near the cushion, but not on the margin. The hairs may reach a length of 2 mm.

3. The wall of the antheridium typically consists of five cells: an oblique basal cell, a lower ring cell with a "binding wall" on one side; an upper ring cell, an opercular cell, and a crescent-shaped cell formed by the division of the cap cell. Many variations from this type occur. The antheridia of *Lophosoria* are larger and less symmetrical than those of *Alsophila*, *Hemitelia*, and *Cyathea*. Those of

Thyrsopteris are the largest and least symmetrical of the family, ordinarily not conforming to the type just given. The antheridia of the Dicksonieae are usually larger than those of the Cyatheae.

4. The necks of the archegonia in the Cyatheae are straight or slightly curved; they have more cells in the neck and are longer than those of the Polypodiaceae. Those in the Dicksonieae and *Thyrsopteris* are longer than those of the Cyatheae, and are more apt to be recurved. The walls of the neck cells are more or less cutinized. The neck contains two neck canal nuclei not separated by a wall. Archegonia with four neck canal nuclei were found in all species.

5. Apogamous growths were found in *Lophosoria quadripinnata*, *Alsophila excelsa*, *A. armata*, *Hemitelia parvula*, *Cyathea medullaris*, *C. dealbata*, *Thyrsopteris elegans*, *Dicksonia squarrosa*, *Cibotium barometz*, and *C. schiedii*.

6. The evidence from the gametophyte indicates that the family Cyatheaceae, in the broader sense, is not a natural group, but is of polyphyletic origin.

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EVIDENCES OF HYBRIDISM IN SELAGINELLA¹

JEANNETTE F. GRAUSTEIN

(WITH FIFTY-THREE FIGURES)

Introduction

The genus *Selaginella* represents a survivor of an ancient group whose former supremacy has lapsed toward extinction. The affinities of *Selaginella* are with fossil *Lycopsidea* of the Paleozoic and a few other present-day remnants, but that it has achieved diversification among the alien *Pteropsida* is suggested by the large and still increasing number of species described. Over 600 species are now recognized, about 50 of which belong to the primitive and polymorphic "*rupestris* group," which includes all the species of *Selaginella* of the United States with the exception of the eastern ranging *S. selaginoides* and *S. apoda*. Many of the species exhibit a polymorphism which has made the taxonomy of the genus a persistent problem.

The chief purpose of this research was the study of meiosis in the microspores of both native and exotic species of *Selaginella*, to determine whether or not irregularities occur, with the hope of obtaining information on the evolution of species in this extensive genus.

RUSSOW (51), one of the early workers on the vascular cryptogams, did not publish his observations on spore development in *Selaginella* because he considered them defective owing to poor and sickly material. HEINSEN (24) studied the megaspore and female gametophyte, but failed to observe any case of mitotic division either in spores or vegetative tissue, and, as he saw elongated nuclei, he concluded that amitotic division was possible. FITTING (18) investigated megaspore development. He found *S. martensii* unsuited to this purpose because many spores developed anomalously. The study of spore formation in all the species was difficult owing to the smallness of the mother cells and the lack of clear nuclear differentiation. HIERONYMOUS and SADEBECK (28) observed that sterile sporophylls occur in many species; that cones often become vegetative;

¹ Contribution from the Laboratories of Plant Morphology, Harvard University.

that branching of cones and even repeated branching occurs; that in some species megasporangia or microsporangia fail completely; and that vegetative reproduction is common and in some species is provided for by special organs. Especially striking was the constant failure of microspores in all investigated cones of *S. rupestris*, whereas the megasporangia inclosed one or two large megaspores of unusual size with two or three small undeveloped spores. They concluded that some form of apogamy obtains in this species. BOWER (6) referred to the vegetative reversion of cones and to the presence of abortive sporangia at the base of cones in *S. selaginoides* and *S. martensii*. GOEBEL (21) demonstrated that self-fertilization is impossible under normal conditions of sporangial dehiscence.

Miss LYON's (40) study of the developing microspores of *S. apoda* showed that a comparatively small number matured. She states: "Curious aberrations in growth are constantly found. . . . Not more than five-sixths of the potential mother cells divide into spores; the others rapidly disappear." In the summary she states that a large percentage of the microspore mother cells form tetrads, "the largest proportion of which abort at this stage." Only a very small number of sporophytes develop. Local material of *S. rupestris* Miss LYON found almost purely megasporangiate. The megaspore mother cells divided variously: either only two daughter cells were formed or one or both divided again, resulting in two, three, or four megaspores; occasionally the mother cell failed to divide and acted directly as a megaspore; also two mother cells might function and form eight megaspores. In any case only one or two spores continued to grow after the early stages. No gametophytic development was seen, and the basal spores differed from the younger ones in size only. Miss LYON considered that this was due to abnormal material until she found sporophytes developing from similar megaspores. It is noteworthy that her figs. 120, 121, and 124 show five "dumbbell" megaspores but no comment was made regarding them. In both species sporophytic development was observed to occur within unshed sporangia. GOEBEL (22) regarded this as an expression of a pathological condition.

Later Miss LYON (41) made a preliminary announcement of apogamy in *S. rupestris*, in a paper which seems to have escaped the

notice of European workers in the genus. She found that embryos are frequently formed from the initial cell of the archegonium, and that until the third or fourth division of this initial it is impossible to determine whether a normal archegonium or an embryo will result.

The only cytological examination of the establishment of spores in *Selaginella* is by DENKE (13). Difficulty in differentiation and the small size of the chromosomes hampered the investigation. All stages of division of the microspore mother cells could be observed in a sporangium whose mother cells had started to divide, and a small percentage of them do not divide. An odd method of spindle formation was observed following the movement of the nucleus to the periphery of the cell. Kinoplasmic threads appeared in the cytoplasm near the nucleus, at first without order; from these delicate threads a bipolar spindle was formed with well defined poles, which continued to grow larger. When the spindle had increased so that its poles almost reached the cell wall, very fine rays were formed from the poles to the nuclear wall, which drew the nucleus (now in prophase) back into the center of the cell onto the spindle. This was the first report of such a spindle formation in plant cells. HERMANN (25) described something comparable in the spermatocytes of the salamander; it has been seen in other animals, and is known as "Hermann's spindle." The heterotypic divisions of the microspore mother cells of the species of *Selaginella* examined were figured as regular; the homeotypic divisions were not studied owing to the smallness of the chromosomes. Attempts to follow the division of the megaspore mother cell were unsuccessful.

MITCHELL (43) and SYKES and STILES (59) discussed the cones of *Selaginella*. In the first paper abnormalities were strongly emphasized; the second pointed out the same conditions: a tendency to reduction of the number of megaspores in various species, frequency of aborted sporangia, and rarity of microsporangia in some species.

BRUCHMANN'S (8) monograph on *S. selaginoides* (L.) Link (*S. spinulosa* A. Br.) noted the low percentage of germination of the megaspores, the small number of archegonia formed, and the absence of development of female prothallia in unshed megaspores. Later (9) he found apogamy in *S. selaginoides* and *S. rubricaulis*. He

was led to investigate the latter because of the very few microsporangia, formed only at the apex of cones sometimes 2 cm. long. All of the megaspores placed on damp blotting paper in a petri dish germinated except very small ones. All embryos were formed behind closed canals with unraised cover cells. There were archegonia in which the canal had opened but the eggs in these never developed; if the cover cells started to arch normally the egg beneath would not form an embryo. In 1919 BRUCHMANN (10) added another apogamous species to those earlier reported, *S. helvetica*, native to Europe, in which practically all archegonial canals remained closed, and beneath those which underwent secondary thickening, embryos developed.

HIERONYMOUS (26) noted two Asiatic species which he suspected of apogamy. Both are widely dispersed in spite of the fact that one (*S. intermedia*) produces no microspores, and in the other (*S. belangeri*) all of the microspores degenerate. Later, working on the taxonomy of material from Australasian islands, he was able to confirm his opinion that many species of *Selaginella* have been able to achieve their otherwise inexplicably wide dispersal through apogamy (27). To species already cited as suspected, he added *S. rugulosa* and *S. longiaristata*. GOEBEL brought back from Brazil *S. anocardia*, a species nearly related to *S. apoda*, which, as it was widely dispersed although practically without microsporangia, he suspected of apogamy, which he was able to demonstrate (23). GOEBEL suggested that apogamy occurs also in *S. apoda*, and that for this reason Miss LYON (40) found megaspores germinating while still in the strobili.

Both BRUCHMANN (9) and GOEBEL (23) believed that there was lack of reduction in the megaspore formation of apogamous *Selaginella* species. This theory was based chiefly on the cytological investigation by STRASBURGER (58) of the reduction divisions of the apogamous *Marsilia drummondii*. This species presents some illuminating parallels to conditions observed in species of *Selaginella*: it was found to be polymorphic to a marked degree; some forms had only sterile microspores; the two accessory canal cells failed to disintegrate and the archegonia remained closed in prothallia capable of developing parthenogenetic embryos, and the spindle was formed in the cytoplasm of the mother cells during prophase. Observations

of meiosis in the megaspore mother cells showed that two kinds of megaspores were developed, as approximately half of them underwent a somatic division, producing megaspores with the diploid number of chromosomes, and about half went through a reduction division which in some cases was regular and in others very irregular. In the microspore mother cells irregularities were so extreme that in most of the forms the first division was never completed. The prothallia developed from unreduced megaspores formed archegonia which maintained closed canals behind which embryo formation took place.

Materials and methods

The material studied consists of six exotic species gathered through two seasons at the greenhouses of the Harvard botanic gardens, and two native species. The greenhouse species are *S. amoena* Hort. (*S. caulescens* (Wall.) Spring) from Asia; *S. emmeliana* Van Geert from Ecuador and Mexico; *S. flabellata* (L.) Spring, universal in tropical and subtropical regions of America, Asia, and Polynesia; *S. kraussiana* (Kunze) A. Br. from Africa; *S. mandaiana* Hort. and *S. martensii* Spring var. *variegata* Hort. Makoy. *S. martensii* is from Mexico. According to HIERONYMOUS and SADEBECK, the variety known as *variegata* is only a sickly garden form with streaked or entirely white portions on some shoots. BAILEY (1) spoke of *S. mandaiana* briefly in a footnote as a recent introduction by W. A. MANDA which cannot be satisfactorily placed. MANDA, a New Jersey seedsman, gave the following information concerning its origin:

When we used to import orchids from different parts of the tropics, we saved all the refuse and trimmings and planted them carelessly under a bench to see if something might come up of horticultural interest, and this *Selaginella* was one result.

The native species examined are eastern ones, *S. apoda* (L.) Fernald (*S. apus* Spring) and *S. rupestris* (L.) Spring. *S. apoda* came from Core, West Virginia, and Pepperell and Granby, Massachusetts; *S. rupestris* from seven regions: Woburn and three different stations on the Mount Holyoke-Norwottuck Range, Massachusetts; Cape Elizabeth, Maine; Berlin, New Hampshire; Bolton, Vermont; Sandy Cove, Nova Scotia; and the shores of Lake Huron.

For the greenhouse species two fixatives were used, Rabl's 0.75

per cent chromo-acetic acid, and Carnoy's fluid; the native species were killed in Carnoy's only. The use of an exhaust pump aided rapid penetration of the tissues. A softening agent (10 per cent aqueous solution of a saturated solution of sodium chlorate in hydrofluoric acid) was used to overcome resistance encountered in cutting the tough spore coats and sporangial walls. The sporangia were then pricked to allow penetration of the nitrocellulose in which the cones were subsequently imbedded. Sections were cut 5 and 10 μ , overstained with Haidenhain's iron-haematoxylin, differentiated, and sometimes counterstained with eosin.

Dividing mother cells occur rarely and great difficulty was experienced in properly differentiating them. Not only are the mother cells surprisingly minute, so that little can be seen under high-power, but their callose coat is of comparatively great thickness, and reflects the light in a water mount to such a degree that usually nothing can be seen but the color of the cytoplasm.

Investigation

IRREGULARITIES IN DEVELOPMENT AND SPORE CONDITIONS

Some abnormalities in the cones were noted. A cone of *S. emmeliana* had split at the summit into two vegetative shoots; a cone of *S. elegans* had bifurcated, and in *S. martensii* var. *variegata* a bifurcated cone appeared in which one of the branches had redivided. Mother cells with two and three nuclei were seen in *S. apoda* and with two nuclei in the variety of *S. martensii*. In most species a number of microspore mother cells failed to divide; in *S. martensii* var. *variegata* this group amounted to half of the mother cells. In this species and *S. emmeliana* an exine coat was laid down about such cells, some of which already showed signs of disintegration, whereas in *S. apoda* this did not occur, but the mother cells which did not divide disintegrated rather promptly.

In *S. amoena*, *S. martensii* var. *variegata*, and *S. mandaiana* collapsed young sporangia are conspicuous. When the collapse is in process the mother cells may appear healthy or the cytoplasm may show vacuolation; the wall may look normal or signs of degeneration may appear in the dehiscing layer or in the tapetum. In *S. emmeliana* the contents of very young sporangia frequently abort; the wall

maintains its position and the two outer layers look normal but the mother cells collapse and the tapetum has an abnormal staining reaction or is absent.

There is a high percentage of abortion in the microspores of all the species, with the possible exception of *S. kraussiana*, which sheds its sporangia so early that observations of spore conditions were curtailed. In fact *S. apoda* is the only species in which any advanced development of the male gametophyte was seen, and only a few spores of any of the species start gametophytic development. Spores of varying sizes and staining capacities appear; there are collapsed, wizened, and large empty spores with perfectly sculptured exine coats. A brownish exine instead of the natural clear yellow is formed about all of the spores in some sporangia. The presence in abundance of small yellow granules in a viscid medium is frequently observed in sporangia, corresponding exactly in color with normal exine coats, and suggesting an abnormal functioning of the tapetum. Undeveloped spores, discolored exine coats, and yellow granules occur also in the megasporangia.

SPINDLE FORMATION

The megasporangia and microsporangia are indistinguishable until the mother cells are formed. When the microspore mother cells are established they round up rather promptly, while growth of the sporangial wall continues, so that they lie freely in a fluid-filled cavity at an early stage. All of the non-functioning cells of the megasporangium fail to round up, and show highly vacuolated cytoplasm, contrasting conspicuously with the densely protoplasmic, dark-staining sphere of the megaspore mother cell (figs. 18, 19). This cell stains darker, is slightly larger, and has a thicker callose coat than the microspore mother cells of the same species.

The method of spindle formation in the megaspore and microspore mother cells is the same in all species, although the phenomenon is more prominent in some species than in others. There is no difference caused in its appearance by the two fixatives used. As the nucleus of the mother cell starts into activity a very fine network of chromatin appears, forming the synaptic knot at the outer edge of the nucleus, which by this time has moved to the periphery and ap-

pears to protrude somewhat from the cell. The spindle fibers apparently develop from some of the cytoplasmic contents, and appear as fibrillae stretching in a broad formation across the cell (figs. 25, 26). They lie on the opposite side of the cell from the nucleus; but later the nucleus returns to the center and is enveloped by the spindle fibers (figs. 11, 12). The different views of spindle formation cannot be interpreted so that they correspond with DENKE's (13) description of spindle poles and a spindle which grows in length. The growth observed is in breadth and no polar points were seen. It may be noted that the Carnoy killing fluid used in this investigation is similar in composition to DENKE's acetic-alcohol fixative.

REDUCTION DIVISIONS

The critical stages of division of the microspore mother cells are of rare occurrence and are found in sporangia whose cells are predominantly in prophase or in tetrads. The divisions are evidently rapid and the mother cells lacking in concerted action. Because of the smallness of the cells, the presence of bodies taking the chromatin stain (which commonly appear in the cytoplasm) is a confusing factor. Since such bodies may occupy positions which might be assumed by the chromosomes it becomes difficult to judge whether one is dealing with chromosomes or what may be called for convenience "chromatin bodies." In some species the conditions are so extreme that no conclusions can be reached.

1. *S. amoena*.—A clear case of a regular heterotypic metaphase was never seen. Many cells in the first division have chromosomes scattered all over the spindle in such a way that one does not know whether to consider the condition metaphase or anaphase, suggesting FEDERLEY'S (16) term "metanaphase." In other cases there is an alignment of some chromosomes at the equator, however, with others scattered on the spindle (fig. 1). There are some metaphases which at first seem perfectly regular, as a black plate of chromosomes stands out sharply at the equator, but closer inspection shows also chromatin bodies of the same size as the univalent chromosomes at each pole, and it is impossible to be sure whether these are chromosomes which have departed prematurely from the equator, or whether they are other chromatin bodies (fig. 7). The narrowness



FIGS. 1-16.*—Figs. 1-8, *S. amoena*, microspore mother cells: 1, irregular heterotypic metaphase; 2, irregular homeotypic metaphase; 3, irregular heterotypic division with univalents and bivalents in abnormal positions; 4, regular homeotypic telophase with chromatin bodies in cytoplasm; 5, heterotypic telophase with chromatin bodies in cytoplasm; 6, tetrad stage with pseudonuclei, one spore has extra true nucleus; 7, heterotypic metaphase with chromatin bodies or univalent chromosomes at poles; 8, tetrad showing heavy callose coat. Figs. 9-16, *S. apoda*, microspore mother cells: 9, mother cell with large chromatin bodies in cytoplasm (attempt at amitotic division?); 10, mother cell showing clearly defined vacuoles in dense cytoplasm (thought by LYON to be concerned in spindle formation); 11, diakinesis with nine chromosome masses, irregular nucleolus, chromatin threads, and small chromatin bodies (spindle appears enveloping nucleus); 12, diakinesis with nine chromosomes, some clearly bivalents, vacuolating nucleolus, and spindle enveloping nucleus; 13, heterotypic metaphase, slightly irregular, with one unpaired chromosome; 14, interkinesis, cell vacuolated; 15, regular homeotypic telophase; 16, homeotypic telophase showing typical sextuple spindles.

* Drawings made with 1.5 mm., n.A. 1.3 apochromatic Zeiss oil immersion objective and a 10X compensation ocular. A cell was drawn with Bausch and Lomb camera lucida and enlarged twice. All figures drawn to this scale except figs. 17-24, which are on a lower scale. Drawings reduced approximately one-half in photographing.

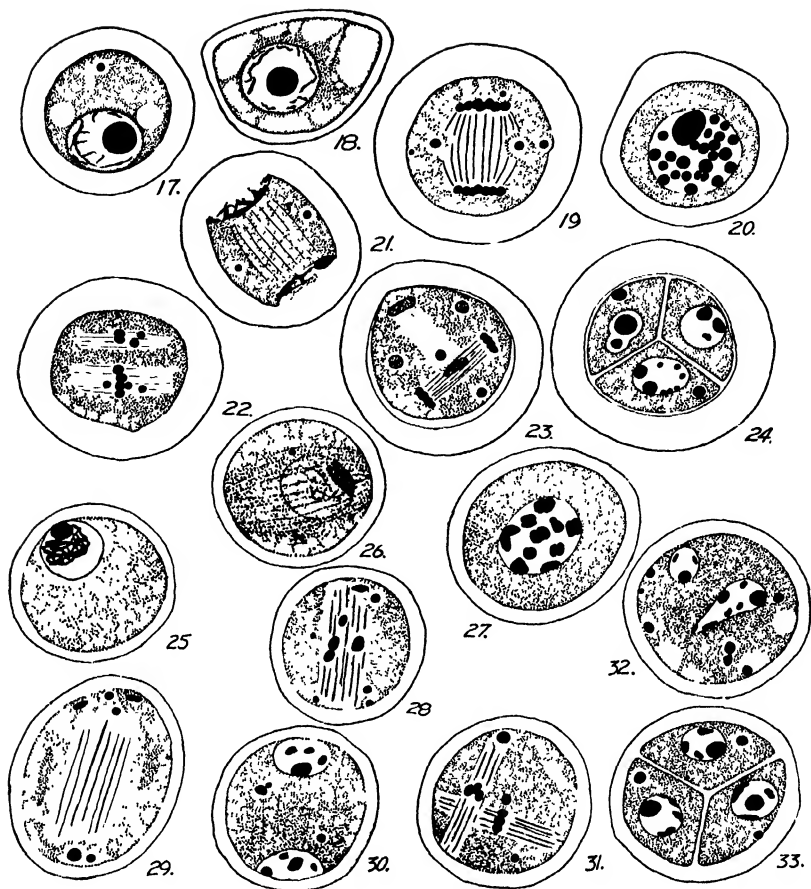
of the plate suggests the former condition. No estimate of the chromosome number could be made. In most of the dividing cells there are varying amounts of chromatin in the cytoplasm, usually near the cell wall. At interkinesis the chromatin bodies appear near the poles or the equatorial plane but always at the periphery of the cell, and a clear zone in the cytoplasm is seen about them; this halo is usually present at all stages but seems especially pronounced at interkinesis and the tetrad stage (figs. 1, 2, 4-6). In fig. 4, at homeotypic anaphase, chromatin bodies are shown lying outside the cytoplasm but within the callose coat; this is comparable with cells noted in *S. kraussiana* (figs. 34, 36), where there is evidently an effort to eject chromatin bodies from the cytoplasm. The homeotypic division is sometimes regular and sometimes not (figs. 2, 4). At the end of this division there are commonly present in the mother cells four nuclei and a number of pseudo-nuclei. However, some cells have only two or three large nuclei; one was observed with one nucleus of normal size and twelve small nuclei, and one had five nuclei of equal size and similar organization besides a number of pseudo-nuclei (fig. 6). In the last case walls had already been laid down, cutting off four spores, which resulted in polycary in one of the spores. No case of polyspory was observed in *Selaginella*, and so far as the writer knows, the formation of more than four microspores in the mother cells of pteridophytes has never been reported, even in forms where extreme irregularities occur during meiosis (31).

2. *S. apoda*.—In this species the chromosomes at diakinesis are commonly distributed about the periphery of the nucleus as well defined entities, and often the bivalency of some of the chromosomes is apparent. In fig. 12 such a condition is shown, with nine bodies, five of which are manifestly bivalent. The nucleolus is vacuolating as is usually the case in late diakinesis. The cell shown in fig. 11 is evidently at an earlier stage, as the nucleolus is budding off chromatin, and three small chromatin bodies appear connected to the chromosomes by delicate chromatic threads. Here again nine masses appear. As some deviations from this number were observed, and as there is reason to doubt the constancy of bivalent formation (since apparently unpaired chromosomes have been seen at diakinesis and metaphase), the writer hesitates to assign a definite chromosome

number to *S. apoda*. The heterotypic division generally shows a slightly irregular metaphase and early anaphase, and sometimes the presence of an unpaired chromosome is evident. While chromatin in the cytoplasm is not a constant feature in this species, it is usually present (figs. 11, 13, 14). Many cells in division and at interkinesis are much vacuolated (fig. 14). The homeotypic division is regular. At the telophase of the second division four additional spindles appear, connecting the four poles of the two division spindles (fig. 16). This appearance is characteristic in *Selaginella*.

Fig. 9 presents an odd condition found in one sporangium. The cell drawn is the most illuminating for the interpretation of the situation, as the original nucleus is readily distinguished and it is evident that the nuclear-like bodies consist of chromatin budded off from the nucleus, each of which has formed a clear space about itself in the cytoplasm. In most of the cells the nucleus has lost more of its chromatin, and five or six seeming nuclei appear; however, one of these is always larger than the others although it does not always show a different organization. Without careful study of all the cells it might be assumed that the meiotic divisions had occurred with irregularities which had resulted in supernumerary and unequal nuclei. In one cell of this sporangium a division spindle appears.

3. *S. martensii*.—Diakinesis is often clear, although sometimes many of the chromosomes are massed against the nucleolus. Only occasionally can bivalency be seen definitely, and probably a considerable number of the chromosomes are univalents. In the cell shown in fig. 20 twenty-four chromatic bodies appear, which is the highest number counted; these all appear to be univalents. In many mother cells chromatin is seen in the cytoplasm before the prophase starts, but the amount is much greater at the anaphase and at interkinesis, when chromatin bodies regularly occupy peripheral positions in the equatorial plane and appear like small nuclei (figs. 17, 19, 21, 23). Metaphases and early anaphases are rarely seen, and no irregularities were observed except in the cell shown in fig. 22, which may be either a heterotypic metaphase with a split in the spindle, or a homeotypic with a difference in size of the spindles caused by a difference in chromosome number. An outstanding feature in this variety is the extreme vacuolation apparent in many cells, especially



FIGS. 17-33.—Figs. 17-24, *S. murtensii* var. *variegata* (figs. 18, 19, megaspore mother cells, other figures microspore mother cells): 17, clearly defined vacuoles in dense cytoplasm; 18, degenerating mother cell from megasporangium; 19, late anaphase with chromatin bodies at equatorial periphery (callose coat is comparatively thicker than in microspore mother cells); 20, diakinesis showing twenty-four chromosomes (spindle inadvertently omitted); 21, interkinesis showing polar region vacuolate and chromatin bodies at equatorial periphery; 22, metaphase, either heterotypic with split spindle or homeotypic with spindles of unequal size; 23, homeotypic telophase with chromatin bodies at equators; 24, tetrad with pseudonuclei. Fig. 25, *S. amoena*, microspore mother cell: early stage of spindle formation. Figs. 26-33, *S. amoena*, microspore mother cells (except fig. 27, which is megaspore mother cell): 26, early stage of spindle formation viewed from point opposite nucleus; 27, diakinesis showing ten chromatic bodies, no nucleolus distinguishable, spindle inadvertently omitted; 28, irregular heterotypic spindle showing chromatin bodies at poles; 29, vacuolated cell at interkinesis showing failure of nuclear formation at one pole; dark bodies parallel to spindle apparently chromatin being absorbed by cytoplasm; 30, interkinesis with chromatin bodies in range of spindle (laggard chromosomes?); 31, homeotypic metaphase with chromatin in cytoplasm; 32, cell at end of second division, probably resulting from one like that in fig. 29; 33, tetrad with pseudonuclei.

at the poles during anaphase and interkinesis (fig. 21). The pseudo-nuclei so prominent at division stages (figs. 19, 21, 23) are often seen within young daughter cells (fig. 24); but they disappear quickly, either by absorption into the cytoplasm or by being taken up by the nucleus.

4. *S. emmeliana*.—At diakinesis the nuclear volume does not enlarge appreciably; the chromosomes are closely crowded together, and often there are small particles of chromatin present, so that attempts to count the chromosomes are unsatisfactory. If there is a nucleolus present it must be very similar in size to the chromosomes. Fig. 27 shows diakinesis in a megaspore mother cell which is somewhat bigger than the microspore mother cells. The bivalency of most of the chromosomes is apparent, but whether one of the ten masses is a nucleolus is doubtful. DENKE gives the chromosome number in *S. emmeliana* as eight.

Usually chromatin bodies are seen in the cytoplasm before prophase begins, and increase continually until at the homeotypic division there is a greater bulk of basic-staining substance in the cytoplasm than there is on the spindle. The cells during division stages assume a muddy appearance, and the spindle fibers take the stain deeply, especially at the second division when sextuple spindles appear, so that differentiation which brings out the chromatin bodies fails to reveal chromosomes on the spindle fibers. Often at telophase and interkinesis there seem to be fine bodies on the fibers. Fig. 28 shows a heterotypic division characteristic of the few seen. One wonders why so few chromosomes appear in these cells and similar ones in other species (*S. kraussiana*, fig. 34). The simplest explanation is that some or all of the chromatin bodies in the cytoplasm and near the poles of the spindle are chromosomes which have been ejected from the spindle. Another possibility is that some of the chromosomes disintegrate into fine bodies which become strung out on the spindle fibers and so cause them to stain darkly. If these gradually become absorbed by the cytoplasm, the dark muddy appearance characteristic of the division stages would be explained.

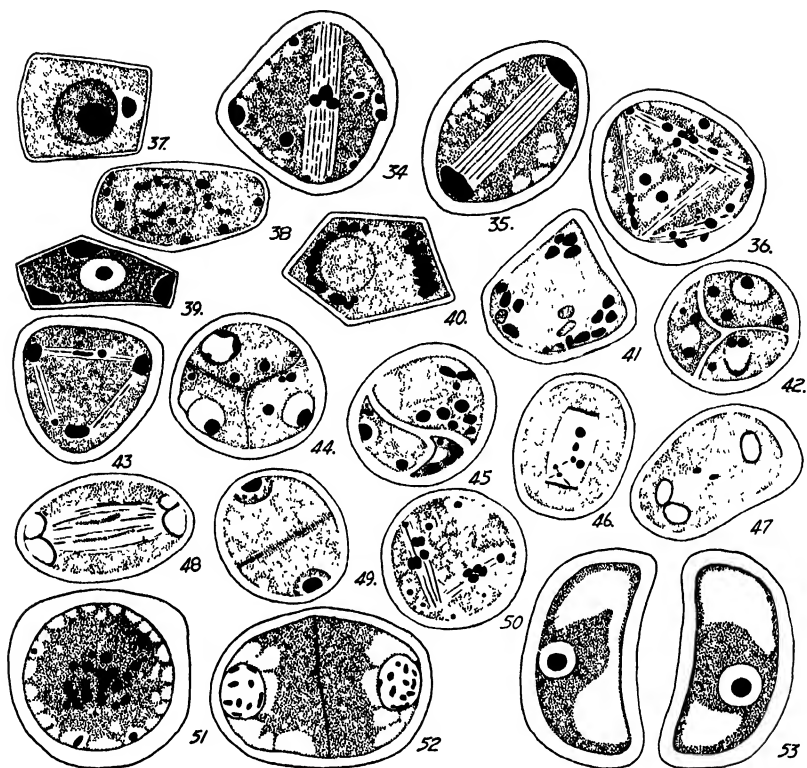
At interkinesis, although there are sometimes two daughter nuclei without any chromatin elsewhere in the cell, the condition shown in

fig. 30 is more common. The small nuclei, instead of being at the periphery of the cell and largely at the equatorial plane (as in *S. martenisii* var. *variegata*), are in the plane and range of the spindle, which suggests that they are formed from laggard chromosomes remaining on the spindle. Fig. 29 shows that a daughter nucleus has failed to form at one pole, and its place is taken by a number of small nuclei. A variation of this condition, in that two nuclei are formed at one pole at interkinesis (neither occupying the central position), is frequently seen. The cell shown in fig. 32 may be the product of such a condition. Explanation of these observations is hampered by the minuteness of the cells and the scarcity of earlier stages. Fig. 29 depicts a condition seen a few times in *S. amoena* and commonly in this species. Elongated bodies, staining grayish blue with haematoxylin, appear in the cytoplasm on each side of and parallel to the spindle in heterotypic telophase and interkinesis. These bodies are probably chromatin which is disintegrating in the cytoplasm. Small nuclei are seen in the tetrad cells; it is believed that at least some are formed by chromosomes.

5. *S. flabellata*.—The mother cells in this species were the smallest seen and the chromosome number seems to be low. A number of divisions were observed, but the extreme size of the figures makes it more impossible than in the other species to determine whether the apparent laggards are really chromosomes or are other chromatin bodies. A number of heterotypic metaphases with neat plates had distinct chromatin bodies of the size of the chromosomes at the poles, very much like the cells in *S. amoena* (fig. 7).

6. *S. mandaiana*.—This species of uncertain origin bears cones in the great profusion characterizing all of the species studied except *S. kraussiana*. Large quantities of the cones abort all their sporangia while in the mother cell stage, however, so that they are visibly empty. Thousands of mother cells were seen in this species in extremely anomalous stages, which can be assigned to two different modes of amitotic division. The more prevalent and remarkable one will be described first.

In this form of division the contents of the nucleus pass out into the cytoplasm, the cell divides somewhat quantitatively, and new nuclei are organized (figs. 37-45). Nuclear activity begins while the



FIGS. 34-53.—Figs. 34-36, *S. kraussiana*, microspore mother cells: 34, heterotypic metaphase showing evident effort to eject some chromatin from cytoplasm; 35, heterotypic telophase with vacuolated cytoplasm; 36, erratic division with malformed spindles, vacuolated cytoplasm, and many chromatin bodies, some ejected from cell. Figs. 37-50, *S. mandaiana*, microspore mother cells: 37, chromatin ejected from cytoplasm formed into large mass with halo, nucleus clearly defined; 38, chromatin in cytoplasm in small masses, some passing through nuclear membrane; nucleoplasm not distinguishable; 39, chromatin massed at three points, nucleolus and nucleoplasm well defined; 40, no chromatin left in nucleus although nuclear membrane persists; 41, chromatin bodies collecting in different peripheral regions, no nucleus; 42, tetrad without sharp 120° angle characteristic of trifacial spores of *Selaginella*; 43, sporadic cell with malformed spindle occurring among mother cells dividing as shown in figs. 37-42; 44, tetrad lacking sharp 120° angle; 45, tetrad walls laid down, nuclei not yet organized; 46, nucleus elongate, chromatin at poles (seen as ring in oblique views) and chromatin masses and chromatin threads toward center; 47, later stage, nucleus more elongate, one polar ring split into two and nucleoplasm hardly distinguishable; 48, dark bodies on spindle interpreted as degenerating chromatin; 49, interkinesis, wall forming; 50, irregular heterotypic division, impoverished cytoplasm. Figs. 51-53, *S. rupestris*, megaspore mother cells: 51, polar view of heterotypic metaphase; 52, wall laid down at end of first division; cytoplasm vacuolate at poles; 53, dyad megaspores.

mother cells still lie closely together in the unexpanded sporangia. This is in striking contrast to normal early meiosis in *Selaginella*, when the rounded mother cells lie freely in the cavity of the enlarged sporangia. Fig. 37 shows a cell with much chromatin extruded into the cytoplasm as a formless mass, the nucleus still retaining chromatin sufficient to distinguish the nucleoplasm from the cytoplasm. In fig. 38 passage of chromatin from the nucleus to the cytoplasm is seen; there is less chromatin in the nucleus than in the cytoplasm, and there is no distinction in the reaction of the two plasmas to the stains. On the other hand, in fig. 39 a cell appears which still shows nucleoplasm although most of the chromatin is in the cytoplasm, massed in three places as distant from one another as possible. In fig. 40 no chromatin is left within the nucleus, but the nuclear membrane is still clear; in the next figure the membrane is gone and the chromatin bodies appear congregated at different points. This chromatin gives no suggestion of being organized as chromosomes. One of the surprising features in these cells is the great quantity of chromatin, apparently far exceeding the volume present in the chromosomes of the normal division. This may be due to differences in physical condition. Fig. 43 shows an erratic spindle formation in a sporangium filled with cells of the general appearance shown in fig. 41. When sporangia were first seen, filled exclusively with cells in the various stages described, it was thought that they were going through a disintegration process and would immediately degenerate. Examination of thousands of sections, however, shows that if this process occurs in a cone all of the subsequently formed sporangia divide their mother cells in the same way, and that abortion of the cells does not occur, at least not until after tetrads have been formed. Figs. 42, 44, and 45 depict tetrads from such cones, all showing a failure of the walls to meet in the sharp 120° angle characterizing the spores of *Selaginella* which are consistently trifacial (figs. 8, 24). The nucleoplasm of two of the nuclei of the tetrad shown in fig. 42 is only weakly distinguished from the cytoplasm by the stain, whereas in the third nucleus there is practically no distinction. Fig. 45 probably is an early stage in the evolution of nuclei from the scattered chromatin, as the focal point of the future nucleus can be recognized in only one cell. The most advanced stage shown is fig. 44

but in this there is still chromatin in the cytoplasm which must be destined either to incorporation in the nucleus or absorption by the cytoplasm. Mother cells in *S. apoda* (fig. 9) seem to have passed through a comparable extrusion and division of chromatin, instead of undergoing the ordinary reduction division.

In cones showing less anomalous conditions the usual early spindle formation was not seen, and the only sign of ordinary prophase was limited to one sporangium. In sporangia showing the second form of amitotic division, about half the cells are in a curious state which suggests both diakinesis and anaphase (fig. 46). The nucleus is elongate and appears somewhat oblong. In this, and in the absence of a nucleolus, the presence of chromatic threads and of dark bodies at the ends of the nucleus, it differs from diakinesis. In some cases the sides of the nucleus and the nucleoplasm are not so clear as in others. In some the dark bodies at the ends are seen to be circular, and are interpreted as aggregations of chromatin at the polar edges of the elongated nucleus, which thereby accounts for the comparatively small chromatin content in the heart of the nucleus. This stage persists for a long time, and its development is not easily traced as subsequent stages are rarely observed. Fig. 47 shows the nucleus more elongate, and so invaded by the cytoplasm that it is almost indistinguishable. The ring of chromatin has formed two rings at one of the poles; these inclose a clear area, and it is evident that from each ring a daughter nucleus will be formed. In some cells two rings appear at each pole. Invariably some chromatin seems left behind in this reorganization. The condition shown in fig. 48 would be considered a later stage of that shown in fig. 47 except for the presence of the spindle, which leaves the matter in doubt. The material in the center of the cell, which appeared as a pale bluish mass, is thought to be disintegrating chromatin which was left behind either in the peculiar direct division just described or in an indirect division. These two forms of division are commonly found associated in the same sporangia.

7. *S. rupestris*.—The cones collected in seven widely separated regions show that this species is almost wholly megasporangiate; from one station fifteen cones were cut in which there were only two microsporangia, and those were both in one cone. Miss LYON (40)

states that microspores are formed in the spring and megaspores in the summer, but the writer saw no indication that the occasional microsporangia are formed exclusively in the spring, or even that this is a usual time for their formation.

Because of the rare occurrence of microsporangia there was no opportunity to observe the meiosis of the microspore mother cells, but some divisions in the megaspore mother cells were seen. The functioning megaspore mother cell is densely granular, stains deeply, and is difficult to decolor. In general in *Selaginella* the prophase of the reduction division is a comparatively prolonged stage, as it is commonly encountered. It is of interest, therefore, that no typical prophases were seen in megaspore mother cells of *S. rupestris*. In two cases where the stage was indubitably one of early meiosis, instead of the expected spireme thread an asymmetrical and elongate nucleus showed lumplike masses of chromatin distributed through it. Fig. 51 shows the heterotypic metaphase from polar view. This is not very illuminating except that it reveals a comparatively high chromosome number in this species, which has significant bearing. The next figure is of an interkinesis, if that term can be used when a wall has been laid down; and division must be considered to be completed in the light of the common occurrence in this species of dyads such as are shown in fig. 53. However, many of the megaspore mother cells proceed through both divisions. Counts of the number of megaspores in sixty-eight sporangia taken from material from three different stations gave the following results: 44 per cent contained two megaspores; 28 per cent three megaspores; twenty-five per cent four megaspores, and 3 per cent six megaspores. Occasionally eight megaspores appeared in a sporangium. Regardless of the number of spores, more than two never grow, and in about half of the sporangia only one spore grows. Such large spores are frequently dumb-bell or kidney-shaped, suggesting an incompleted nuclear division which would result in a diploid supply of chromosomes. No gametophytic development was observed, although many are large and old.

The microspores in this species proved to be of great interest. The dumb-bell shape is often encountered, and occasionally bizarre forms. Usually all the spores are full of food and seem to be developing, but advanced stages were not seen. It was continually noticed

that besides being surprisingly large, the spores apparently lay in pairs. To check this, a microsporangium was pricked out in water on a slide where the general occurrence of dyad microspores was unquestionably demonstrated. The spores were commonly tightly associated in pairs, and when they lay separately the markings indicated that two spores had lain together when the first layers of the exine coat were deposited. No triradiate markings were seen, but a few cases were observed where a spore had two or three small collapsed spores clinging to it. As in the sections of imbedded material, the spores were large and elongate, as would result from one division of a mother cell. The significance of dyad formation will be considered in the discussion.

Discussion

Since the days of LINNAEUS, FOCKE (19), and KERNER (35), natural hybrids have been recognized by taxonomists and have provided a subject of slowly growing interest. Doubtless the increased knowledge of natural hybrids is to be correlated with more intensive studies within a group or a definite region. Thus BRAINERD'S (7) work on natural *Viola* hybrids of North America described eighty-two formed among thirty of our seventy-five species. He concluded that hybridization is limited only by failure in cohabitation of species. Hybrids have been found among the pteridophytes, both in nature and in culture. BENEDICT (3) cited fifteen crosses among six native species of *Dryopteris*.

STERILITY OF HYBRIDS.—From the time of KÖLREUTER (36), sterility has been recognized as a conspicuous characteristic of most hybrid forms, although there are hybrids which are fertile. The persistent presence of sterile pollen in a species growing under normal conditions is now generally regarded as indicative of the impurity of the line. HOAR (29), STANDISH (57), and COLE (12) found in pollen sterility indications of widespread hybridism in *Rubus*, *Crataegus*, and *Rosa* respectively, conclusions later verified by the more detailed cytological and genetical observations of PEITERSEN (46), LONGLEY (38, 39), PENLAND (47), BLACKBURN and HARRISON (4), and TÄCKHOLM (60).

REDUCTION DIVISIONS IN HYBRIDS.—Although in the early years of cytological investigation there was an idea current that only

irregular meioses occurred in hybrids, and that the consequent variability in chromosome distribution was the exclusive cause of hybrid sterility, further research has shown that not all hybrids show abnormalities in the reduction divisions. If the parental chromosomes are equal in number the pairing may take place neatly and the divisions proceed with regularity; however, the pollen is usually largely sterile. Irregular distribution of chromosomes at the reduction division has been described for great numbers of hybrids. In some forms all of the chromosomes appear paired at diakinesis, but in most forms which have parents with differing chromosome numbers a definite number of bivalents and of univalents appear. The bivalents divide normally; the univalents may remain undivided in the first division or may split. The classic example of the first type of univalent action is the hybrid between *Drosera longifolia* (n-20) and *D. rotundifolia* (n-10) examined by ROSENBERG (48), in which the unpaired chromosomes at the heterotypic division pass without order to either pole, lag behind so that they form small nuclei, or are left in the cytoplasm where they disintegrate. GEERTS (20) found the same chromosome action in a cross between *Oenothera lutea* (n-7) and *O. gigas* (n-14). SAX'S (53) triploid *Triticum* hybrids followed this scheme in meiosis, but in his pentaploid hybrids the univalents split at the heterotypic plate after the division of the bivalents, resulting in spores whose chromosome numbers varied in a comparatively wide range. This last phenomenon also appears in many species of *Rosa*, whose cytology was studied by BLACKBURN and HARRISON and more extensively by TÄCKHOLM.

In extreme cases there is variable pairing of chromosomes or none. FEDERLEY (16) raised *Pygaera* hybrids, and found that there was no pairing, and both divisions of the spermatocytes were equational. Most of the cells showed anomalous conditions and "pathological appearances." WOLSEDALEK (66) found the cause of sterility in the mule in the destruction of the primary spermatocytes through many forms of degeneration in reduction processes: synizesis was lacking; pairing was very variable; spindle formation was bizarre; giant cells appeared; in some cells of ordinary size the abnormal number of chromosomes suggested that the univalents had split, and attempts of cells to eliminate chromatin were apparent. JEFFREY and HICKS (31, 32) investigated meiosis in *Drosophila melanogaster* in an at-

tempt to gain some light on the origin of its "mutating" power. Both divisions showed the remarkable condition of the complete absence of a metaphase plate, although there were some paired as well as single chromosomes distributed widely on the spindle. The chromosome number was higher than expected; there was much lagging; and a large amount of chromatin was ejected into the cytoplasm. They concluded that these aberrancies were characteristically hybrid.

APOGAMY.—ROSENBERG'S studies (49, 50) on the highly polymorphic and apogamous genus *Hieracium* have thrown much light on hybrid cytology and the allied problems of apogamy, polymorphism, and sterility. OSTENFELD (45) worked on the genetical side of the genus, and furnished pedigreed hybrid material for investigation. ROSENBERG reported that the pollen mother cells in different species and hybrids acted differently: many followed the "*Rosa* type" of reduction and formed pollen with varying chromosome numbers; in others there was no pairing of the chromosomes, so that the interkinesis nuclei received very different numbers of chromosomes. A variation of this consisted in the failure of the chromosomes to pass to the poles at heterotypic division, so that the nuclear membrane was reestablished about the univalents and a "regression nucleus" formed, and dyads with the somatic number of chromosomes resulted from the homeotypic division. Most of the apogamous *Archieracia* species were found to be triploid, and followed the course last described in the division of the pollen mother cells and the embryo sac mother cells.

WINGE (65) and ERNST (14) reported that apogamy was a result of hybridization. Later ERNST (15) reviewed the situation, and pointed out that apogamy is linked with high chromosome number, polymorphism, and poor and impotent pollen. It is rather generally conceded now that hybridization and apogamy are at least related factors. SHARP (55), in discussing ERNST'S theory, expressed the opinion that "little doubt can be entertained regarding the essential correctness of the theory."

AMITOSIS.—ROSENBERG (50) was of the opinion that many of the descriptions of amitosis in reduction divisions which appear in the literature should be ascribed to the formation of "regression nuclei" at interkinesis, resulting from failure of the chromosomes to pass to

the poles, as he described in certain species of *Hieracium*. YASUI (67) and LJUNGDAHL (37) recognized this origin of such figures in the microspore mother cells of poppy hybrids, as BORGSTAM also did in *Syringa chinensis* (5). Other cases of its appearance in microspore mother cells which may confidently be referred to the same source, especially as dyads commonly resulted from such cells, are the findings of SHIBATA and MIYAKE (56) in *Houttuynia cordata*, OSAWA in *Taraxacum albidum* (44), and TOKUGAWA and KUWADA in varieties of garden canna (63).

Reports of division at all suggestive of the amitotic methods described in *Selaginella mandaiiana* have appeared rarely in the literature. CANNON (11) described some "amitoses" in hybrid cotton plants, which in their most degenerate phase suggest, if not the extreme condition in *S. mandaiiana*, at least the somewhat similar one in *S. apoda*. WODSEDALEK (66) showed in his fig. 47 a degenerating primary spermatocyte in the mule, which appears like the more aberrant type of amitotic division in *S. mandaiiana*, although its history is different. In this cell many of the chromosomes have fused into large spherical bodies which remain scattered in the cytoplasm. It should be emphasized that in these cells of the cotton plant and the mule death is imminent, and this is also the case in the tapetum of a *Ribes* hybrid described by TISCHLER (61), where the nuclei lost almost all their dark-staining substance which lay as elongated bodies in the cytoplasm. Oligopyrene and apyrene sperms, which suffer loss and degeneration of chromatin, are not considered to be functional. WARREN (64) examined the spermatogenesis of certain spiders and found surprising conditions. In the primary spermatocytes the spireme broke down into granules, which either divided into small clusters (each of which became surrounded by cytoplasm so that multispermatic formation resulted), or the chromatin granules condensed and formed a number of large masses of inconstant size and number, which resembled chromosomes (each of which became a center for spermatid formation). WARREN thought that the sperms formed by these processes were functional, as they were the exclusive method of spermatid formation in some species.

CHROMATIN IN CYTOPLASM.—Interesting comments upon the occurrence of chromatin in the cytoplasm are made by TISCHLER (62) in the banana, OSAWA (44) in *Taraxacum*, SEARS (54) in *Taraxacum*, LONG-

LEY in *Rubus* (38) and *Crataegus* (39), TOKUGAWA and KUWADA (63) in canna, and FISK in *Zea mays* (17). In many cases where no mention of them is made in the text, the figures show dark-staining bodies in the cytoplasm. JUEL (33), studying meiosis in the hybrid *Syringa rothamagensis* (*S. chinensis*), found copious chromatin in the cytoplasm at the periphery of the cell, which first appeared at diakinesis and later increased in amount. At the end of the second division these chromatin bodies became rounder, lay strewn through the cytoplasm, and appeared to form small nuclei. He suggested that the chromatin may represent the extruded chromosomes from one parent gamete, whereas those of the other divide on the spindle.

Chromatin in the cytoplasm at the divisions of the microspore mother cells in such large amounts as to be a source of confusion has never been reported, so far as the writer knows, in any plants which are not hybrids, or whose purity of inheritance cannot reasonably be suspected. This may be due to a fundamental disturbance in the metabolism of the cells, or incompatibility of chromatic substances; its origin is doubtless in a mixed inheritance.

POLYMORPHISM.—That polymorphism has resulted within genera whose species hybridize freely has been shown in many groups: *Salix*, *Betula*, *Quercus*, *Rubus*, *Crataegus*, *Rosa*, *Viola*, *Aster*, *Solidago*, *Taraxacum*, *Hieracium*, many of the ferns, grasses, sedges, orchids, etc. Moreover, within some "species" great variability is found, so that they come to be regarded as collective species composed of different races, as *Marsilia drummondii* and *Taraxacum officinale*. Such cases of microspecies are commonly associated with apogamous embryo formation. TÄCKHOLM (60) thought that the apogamous rose forms are F_1 hybrids from the Pliocene era, which have continued to the present day through asexual reproduction. HOLMGREN (30) pointed out that species which are facultatively sexual, like some species of *Hieracium*, can continue to cross and produce new forms which may become fixed by apogamous reproduction.

INFLUENCE OF ENVIRONMENT.—Various investigators have experimented with the influence of external agents on cell division. SAKAMURA (52) treated anthers of *Vicia faba* with chloral solution and obtained abnormalities in division which were very similar to

those found in hybrids, mutants, and apogamous plants. By subjecting shoots to low temperature BORGSTAM (5) produced meiotic irregularities in the hybrid *Syringa rothamagensis* (which otherwise was found normal, although JUEL and TISCHLER had found typical hybrid abnormalities in it), and MICHAELIS (42) increased the amount of abnormalities in *Epilogium angustifolium*, whereas STRASBURGER (58) found that diploid eggs developed in great numbers in *Marsilia drummondii* only at high temperatures. Adverse environmental conditions increased the percentage of formation of tetraploid gametes in KARPECHENKO's intergeneric hybrids, *Raphanus sativus* × *Brassica oleracea* (34). Some of these experiments indicate that hybrids are particularly susceptible to adverse conditions.

In considering the influence which the artificial greenhouse environment may have had on spore formation in the exotic species of *Selaginella* examined, it is significant that all the conditions and anomalies found parallels among native species; even the extreme mode of amitotic division frequent in *S. mandaiana* was observed in *S. apoda*. Hence if the greenhouse habitat exerted any influence it was of degree and not of kind.

Conclusions

By virtue of its great antiquity, *Selaginella* may reasonably be supposed to have had all the time and opportunity necessary for the development of great numbers of species by the evolutionary processes. That it has also increased and diversified its members through hybridization seems a necessary conclusion, in view of certain characteristics of many of the species which have been gradually coming to light. Adaptations for vegetative propagation, vegetative anomalies, luxuriance, polymorphism, apogamy, dyad spores, absence of microspores, sterile spores, irregular meiosis, polycary, pathological conditions in the tapetum and dehiscing layers, wholesale extrusion of chromatin in spore mother cells, much chromatin in the cytoplasm of the dividing spore mother cells, failure of mother cells to divide and lack of concerted and consistent action among them: all these conditions have been found in known hybrids, and various combinations are found in many species of *Selaginella*. That all these features are exclusively those of hybrids is not contended, but

when they are concurrently present the evidence is as decisive as may be.

Some of the apogamous species of *Selaginella* must be very ancient hybrids, as they include in their numbers *S. selaginoides*, the species which consensus of opinion has given the most primitive position in the genus, and another nearly related and comparatively simple form, *S. rupestris*. The first of these has the widest distribution of any species of *Selaginella*, and forms of the polymorphic *S. rupestris* are scattered liberally over the western hemisphere and also appear in the eastern.

Natural hybridization among plants as a source of species formation is proving to be of general occurrence. To the long list of groups in which it has been found to be widespread, the Selaginellaceae must apparently now be added.

Summary

1. Some aberrancies of habit were observed, such as bifurcated cones and reversion of the cone at the apex to a vegetative condition.

2. The method of spindle formation in both megaspore and microspore mother cells is very different from the usual one in plants. The spindle fibers appear in the cytoplasm while the nucleus, in synapsis, is at the periphery of the cell. Later the nucleus returns to the center of the cell and is enveloped by the broad spindle.

3. The mother cells are lacking in concerted action and many fail to divide.

4. The study of meiosis is difficult for three reasons: the scarcity of divisions; the extreme smallness of the mother cells; and the quantity of chromatin-like bodies frequently found in the cytoplasm, which in many instances cannot be distinguished from chromosomes.

5. In *S. amoena* the heterotypic division is irregular.

6. In *S. apoda* the heterotypic division is often slightly irregular.

7. Two aberrant types of division are predominant in *S. mandaiana*: (1) the contents of the nucleus pass out into the cytoplasm, the cell divides somewhat quantitatively, and new nuclei are organized; (2) the nucleus elongates and the chromatin is divided without chromosome formation.

8. *S. rupestris*, which is apogamous, is almost exclusively mega-

sporangiate, has a comparatively high chromosome number, and forms its microspores in dyads like many apogamous angiosperms.

9. There is a large percentage of sterility in the spores of the species of *Selaginella* examined. Collapsed microspores, microspores devoid of contents but with perfectly sculptured exine coats, megaspores and microspores with discolored exine coats, small granules of exine material scattered through sporangia, and collapsed sporangia are common features.

10. No advanced stages of development of male gametophytes were seen except in occasional spores of *S. apoda*.

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STUDIES ON THE MORPHOLOGY OF THE ONAGRACEAE

II. EMBRYONAL MANIFESTATIONS OF FASCIATION IN CLARKIA ELEGANS

DONALD A. JOHANSEN¹

(WITH FIFTY-TWO FIGURES)

Introduction

The literature of botany abounds in descriptions of teratological seedlings, cotyledonary aberrations, and similar phenomena (13). It seems, however, that practically all observations have been made on nearly mature plants, *after* fasciations as such became visible. From the observation of germinating seeds of various Onagraceae during the past few years, it has become increasingly apparent that many of these phenomena had their origin during the early development and organization of the embryo. This paper is intended more as a contribution to the study of the origin of fasciation phenomena than as an attempt to explain these anomalies.

In *Clarkia elegans* Dougl. fasciation is a phenomenon commonly observed in pedigree cultures conducted over a period of many years, apparently being carried over from one generation to the next. It is still uncertain with what this heritable tendency is linked or otherwise correlated genetically. According to experiment garden records, compiled over a period of several years, those cultures which possess a high proportion of fasciation show in their seedlings a correspondingly high percentage of cotyledonary abnormalities. Since the cotyledons of many onagrad are decidedly characteristic of the species, and of certain genera as well, any variations from the normal are observable as soon as the modified leaves bearing the cotyledons become fully expanded. In itself, however, this is hardly an infallible sign of approaching fasciation, for the first definite external indications of this teratological condition become noticeable in irregular phyllotaxy preceding the appearance of the inflorescence. It is therefore extremely likely that genes other than those primarily responsi-

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ble for fasciation are the basic cause of simple cotyledonary anomalies.

In 1925 I happened to collect, for embryological study, a number of ovaries from a strongly fasciated plant of *Clarkia elegans*. When sectioned and examined two years later, considerable irregularity in the succession of divisions in the young embryo was noted. This strengthened the supposition just expressed, and suggested that the genesis of fasciation, whether it is to become manifest shortly after germination or during the development of the inflorescence, was to be found in the embryo, even during the formative stages preceding growth of the cotyledons. Further, since the time of SACHS, it has been assumed by plant physiologists that fasciations may readily be produced artificially by mechanical injury, provided such stimulus be applied immediately after germination and that it affects only the initial meristem. If such be the case, would it not be probable that in the ordinary course of events something might occur in the zygote which would so disturb the rhythm of the regular sequence of divisions that fasciation and other abnormalities would become apparent after germination? Preliminary observations on the 1925 material showed that this could readily happen, and consequently it was thought worth while to undertake a special study of the early embryogeny of plants from certain pedigrees long known to possess a decided tendency toward both teratological cotyledonary phenomena and fasciation.

This study has been made on material collected principally during 1928 from the cultures of Dr. L. L. BURLINGAME at Stanford University, who very generously placed his plants at my disposal.

Material and methods

An entire series of ovaries, representing all stages in the development of the embryo, were collected several times over from specially selected individuals in many pedigrees. The most satisfactory killing fluid was that of Petrunkewitsch; the ovaries were cut into convenient lengths, the air removed by means of a hand pump, and after being imbedded were soaked in water for several days and sectioned longitudinally at 10 μ .

OVULE AND MATURE MEGAGAMETOPHYTE

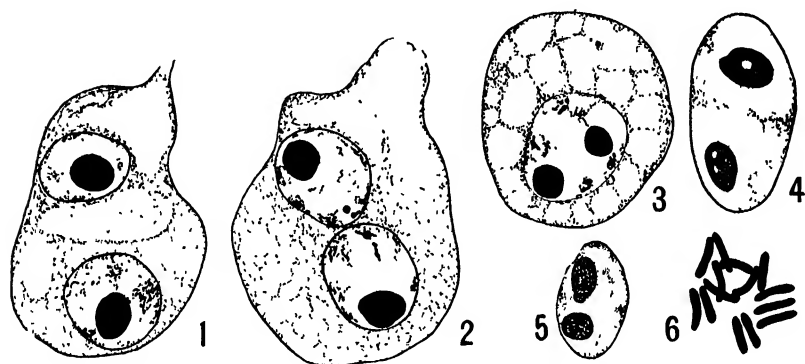
Ovaries from fasciated branches are always shorter and broader than normal ovaries, and the ovules are somewhat smaller. There is no reduction in the number of ovules, consequently the seeds acquire a sharper angularity from mutual pressure. Perhaps on account of the greater supply of water present in fasciated branches, the hypostase (8) is much reduced; an epistase has not been observed, except in a single anomalous instance. No irregularities were noted in the development and organization of the megagametophyte, which, at maturity, is of the regular tetranucleate type characteristic of the family. It is somewhat smaller, however, and occupies less space in the embryo sac cavity. Moreover it is apparently a very fragile and unstable structure physiologically, as it collapses and degenerates with comparative ease and frequency.

FERTILIZATION

For some reason not completely understood, fertilization proceeds unusually slowly in the ovules of fasciated plants of several other onagrads besides *C. elegans*. It is consequently easy to follow the various stages in the entrance of the microgametophyte and the union of the two male nuclei with the egg nucleus and the polar nucleus respectively (figs. 1-3).

In most published figures of onagrad megagametophytes the egg cell appears as if it were a perfectly rounded sphere; at any rate, one scarcely fails to acquire the impression that the egg cell is completely inclosed by a wall or membrane. Such is hardly the fact; in each of over fifty species whose egg cells were examined the cell was found to be pyriform, with the narrowed neck pointed toward and between or over the synergids. The neck is either open or superficially closed by an extremely thin membrane. The male nucleus invariably enters through this opening or through the easily ruptured membrane: no other mode of entrance was observed. At first the male nucleus is surrounded by a thick mass of cytoplasm, part of it coming from the microgametophyte and the remainder from the synergid or synergids demolished during the entrance of the former, but this alien cytoplasm becomes almost wholly divested from the male nucleus in the

upper part of the egg cell, where it mingles with remnants of the egg cytoplasm displaced from below by the entering male nucleus (fig. 1). This commingled cytoplasm is doubtless shut away from the lower part of the cell after the first zygotic mitosis, but it is within the realm of possibility that some of it is brought into the lower portion of the egg cell, and that it may have an effect upon the future behavior of the zygote.



FIGS. 1-6.—Figs. 1-3, syngamy: fig. 1, entrance of male nucleus, bringing plasm from microgametophyte and demolished synergids; fig. 2, later stage; vacuole in upper part of egg (still visible in previous figure) has disappeared; fig. 3, fusion of nucleoplasm complete; nucleoli still distinct; fig. 4, union of secondary male nucleus with polar nucleus, illustrating normal type; dissolution of nuclear walls nearly complete; fig. 5, type in which male nucleus resembles spermatozoid, showing male nucleus fusing first with smaller polar nucleolus (visible as "bud"); fig. 6, chromosomes of first zygotic mitosis; $\times 1250$.

The male nucleus is but slightly smaller than that of the egg; in fact, the two often cannot be distinguished except by the relative position in the egg cell, the nucleus of the latter being generally the lower. The two sexual nuclei are both in the reticulate condition during the entire course of the fusion process; no metabolic changes were observed or have been recorded by others (fig. 2). The union of the nuclei (in these fasciated plants only) may be described as a leisurely process; the male nucleus slowly approaches that of the egg and syngamy proceeds deliberately. After the nuclei become well pressed together, the walls separating them are slowly absorbed into the nucleoplasm of the fusion nucleus (fig. 3). The nucleoli remain distinct for some time, finally fusing. Most writers agree that the

nucleoli fuse, but RENNER (15) contends that in *Oenothera lamarckiana* one of the two "dann zugrunde gehen könnte."

The fact should be emphasized that syngamy in *C. elegans* consists of two distinct phases: fusion of the nucleoplasms first, followed by fusion of the chromatin-containing nucleoli. Furthermore, infinitesimal particles of foreign plasm are rather certain to be adherent to that part of the wall of the male nucleus absorbed into the fusion nucleus. These facts are significant.

The secondary male nucleus is ordinarily similar to the primary male nucleus, and fuses with the polar nucleus while both are in the reticulate condition. The polar nucleus is sister to that inclosed within the egg and contains the haploid number of chromosomes. In nine cases out of ten the polar nucleus is binucleolate, no matter whether the plant from which the ovaries were secured was teratological or not. The size of the smaller of the two nucleoli ranges from 5 to not more than 20 per cent of the bulk of the larger, and it is usually oriented in the upper portion of the nucleus toward the egg cell. It generally fuses with the larger previous to the union of the nucleus with the secondary male nucleus. As in the egg cell, the nuclei first fuse, followed by fusion of the nucleoli, but both processes are consummated with far greater rapidity (fig. 4). Immediately there is initiated a series of simultaneous rapid divisions of the primary endosperm nucleus so created, the successive generations of nuclei gradually diminishing in size until they become mere fragments of chromatin and division is no longer possible. The "endosperm" finally disintegrates completely and no trace of it can be found in mature seeds.

In some instances the secondary male nucleus assumes an entirely different character, possessing the same size, shape, and staining reactions as the nucleolus of a normal nucleus. It was not possible, in many hundreds of ovules, to follow the progress of the nucleus from its entrance into the embryo sac to the vicinity of the polar nucleus, hence exactly what occurs to cause its changed character is not known. In a number of undischarged microgametophytes only normal male nuclei were observed. In any event, the behavior of this nucleus after reaching the polar nucleus has been traced closely: it penetrates the nucleus, fuses first with the smaller nucleolus if the

latter has not already united with the larger, and then with the larger, after which normal division occurs (fig. 5). The number of nuclear divisions subsequently taking place is approximately half the normal, owing to the obvious fact that the bulk of the primary endosperm nucleus is less than half that of a normal one. It had been recognized for some time that in many embryo sacs there was half the quantity of "endosperm" nuclei and cytoplasm as in others: the unusual behavior just described furnishes a satisfactory explanation of this phenomenon.

This is admittedly a most peculiar mode of behavior, and one which apparently has not been reported for any other onagrad. Its significance is probably indicative of a further stage in the gradual reduction in importance of the "endosperm" in that order of the Dicotyledoneae (Myrtales) under which the Onagraceae are placed, and further brings out the relation of the male nucleus to this reduction. Another significant fact is that embryo sacs with well developed normal embryos but lacking every vestige of "endosperm" are frequently encountered in material from both normal and fasciated plants.

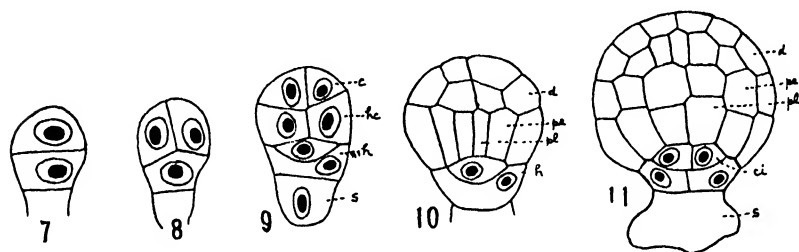
The time elapsing between pollination and fertilization averages 36 hours in normal plants and 44 hours in fasciated plants. The reason for this 8-hour difference requires further investigation.

NORMAL EMBRYONAL HISTOGENY

It was naturally necessary to investigate first the development of the normal embryo in the species under discussion, in order to ascertain if the sequence of divisions was regular, and to provide a basis for comparison. For convenience, the detailed histogeny of the embryo may be given, as worked out by SOUÈGES (16) for *Oenothera biennis*, and confirmed by JOHANSEN (7) for *Godetia amocna*. The development of the embryo and its tissues from the zygote onward is a remarkably uniform process among all species of the family so far investigated.

Following the primary transverse division in the zygote there occurs a longitudinal segmentation of the apical cell, then a transverse partitioning of the basal cell. The two juxtaposed upper cells are destined to give rise to the cotyledons, stem apex and hypocotyl, the

intermediary cell becomes the hypophysis, while the lowest forms the suspensor (fig. 7). The two upper cells divide once longitudinally (at right angles to the first wall) to form the quadrant stage (fig. 8). A transverse segmentation of each quadrant initiates the octant stage (fig. 9). The four superior cells of the octant correspond to the cotyledonary part of the embryo (*c*); the four inferior cells represent the hypocotyledonary portion (*hc*). During the differentiation of the octants the lowest cell (*s*) divides into two which generally do not divide again, although sometimes the lowest increases so greatly in size that it appears hypertrophied. These two cells constitute the suspensor (fig. 39). In several species, although somewhat rarely in



FIGS. 7-11.—Histogenesis in onagrad embryo as illustrated by *Godetia amoena*; $\times 450$.

C. elegans, the suspensor becomes separated from the body of the embryo and withers (fig. 44). There is considerable difference among the species regarding the time at which the hypophysis cell (*h*) divides. Although this statement should not be regarded in any sense as dogmatic, the hypophysis usually divides at the same time that the octants are being organized. The partition is peculiar in that it is concave from above and separates the original cell into two unequal cells; the latter, by means of two vertical divisions, give rise in turn to two groups of four superposed cells (fig. 11 *ci*). In several cases the hypophysis cell had at the beginning two rectangular partitions separating four "hypophysis quadrants" which, by horizontal segmentation, contribute in the same way to the two groups of four superposed cells. The upper group of four cells represents the initials of the cortex (*ci*); the lower group, by vertical, then tangential partitions, gives rise to the two primary layers of the root cap. This last

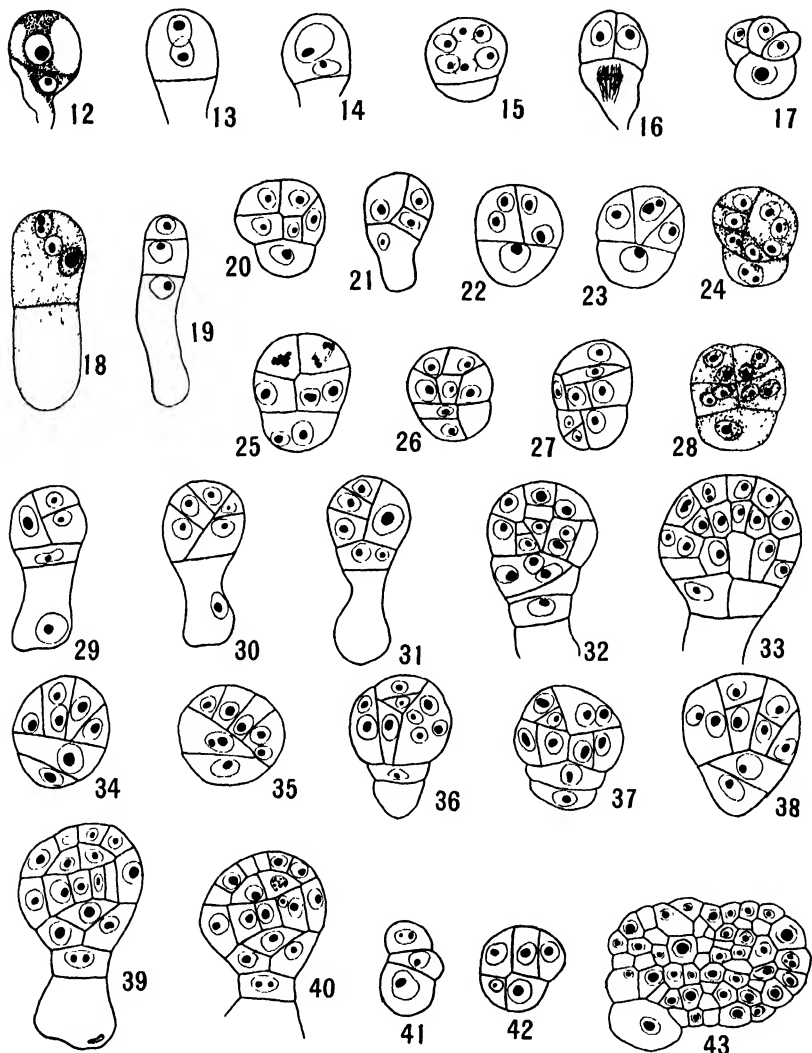
tissue becomes extended to the right and left by tangential segmentations of the dermatogen (*d*) of the hypocotyl axis, and increases in thickness by centripetal divisions of the innermost layer, becoming the calyptrogen layer. The outer layer of cells covering the embryo at this stage constitutes the dermatogen, the next inner group forms the periblem (*pe*), while the innermost gives rise to the plerome (*pl*). Further development proceeds in the usual method characteristic of the Dicotyledoneae. SOUÈGES truly concludes that "it is the precocious differentiation in the hypophysis cell which sharply differentiates the embryo of *Oenothera*."

ZYGOTE AND YOUNG EMBRYO

Owing to the immense number of buds on fasciated plants and to the slowness of fertilization, not more than 80 per cent of the ovules in any particular ovary are fertilized. Nevertheless, zygotes are always found in abundance; the comparatively low proportion of unfertilized ovules in fasciated material to the high percentage of degenerated megagametophytes in normal ovules makes zygotes easier to find in the former. The first postsyngamic mitosis was twice observed (fig. 6); fourteen chromosomes were easily counted in each (in regard to the number of chromosomes in *C. elegans*, see JOHANSEN 9). In a few cases the zygote nucleus had a distinctly amoeboid appearance, consisting of a large, irregularly outlined central portion from which a few broad protuberances extended to the periphery of the nucleus. On the whole, the zygotes and the first mitosis therein show no appreciable difference in comparison with strictly normal zygotes; it is immediately after the primary mitosis that irregularities first become apparent. A certain proportion of the zygotes (ranging from one or two in each ovary to 75 per cent of the total number, depending upon the pedigree) possess two nuclei (figs. 3, 4).

Binucleate (or rarely trinucleate) cells are one of the principal characteristics of embryos from fasciated plants of *C. elegans* (figs. 22, 25, 32, etc.). How well this holds for other onagrads cannot be stated at present, as there has not been the opportunity of collecting fasciated material in sufficient quantity from other species.

Although so far as could be ascertained the first wall in the zygote is transverse, as is invariably the case in normal fertilized egg cells,



FIGS. 12-43.—Fig. 12, failure of nucleus to divide following primary zygotic mitosis; plasmolysis and beginning of degeneration (common occurrence); figs. 13, 14, nuclei retained in upper cell following primary zygotic mitosis; fig. 15, free-nucleate unicellular embryo; fig. 16, apparently normal embryo, with mitosis ($14 [= 2n]$) chromosomes in suspensor cell; fig. 17, irregular young embryo; figs. 18, 19, filamentous embryos, first being free-nucleate (note difference in size, although both embryos came from same ovarian locule); figs. 20-38, various stages in growth of anomalous embryos (cf. suspensors of figs. 29-31 with those of figs. 34-38); figs. 39, 40, normal embryos from strongly fasciated plants; figs. 41-43, stages in development of unilateral embryo; $\times 450$.

the wall is often formed in such a manner that both nuclei are retained in the cell destined to form the embryo proper, thus leaving what should be the initial cell of the suspensor without a nucleus, such a zygote constituting the binucleate form previously mentioned. Between the two a wall may later be formed in the cytoplasm; the upper cell is the embryo initial, while the lower in later behavior simulates the appearance of a suspensor, but is otherwise ontogenetically indistinguishable from the embryo proper. It is quite possible that the irregular suspensors of embryos such as that shown in fig. 36 arose from a cell of the peculiar origin so described. The second division is vertical as usual, but is hardly ever exactly perpendicular to the primary wall; instead it is more or less oblique and usually considerably to one side of the median line (fig. 22). Often a second vertical partition, close to the first at the base but far apart at the periphery, is formed shortly after the first (fig. 23), and sometimes three or more walls may be erected in the upper cell (figs. 34, 35). Generally speaking there is a conspicuous absence of order, sequence, and direction in the formation of all cell walls except the first transverse partition. It is rare that any semblance to normal order is attained (fig. 33); at any rate there are in such cases appearances which stamp the young embryo as teratological, the most decisive of which is the presence of binucleate cells. Strictly normal embryos are always to be observed in ovaries from fasciated plants (figs. 39, 40).

UNILATERALITY AND SPURIOUS MONOCOTYLEDONY

Seedlings with only one normal cotyledon, and possessing or lacking the rudiments of the second, are frequently found in large batches of seedlings, and it was consequently not surprising to find abundant evidence of the origin of this condition in developing embryos. These embryos are especially interesting in view of their possible relation to the controversy as to whether monocotyledons were derived from dicotyledons, or vice versa.

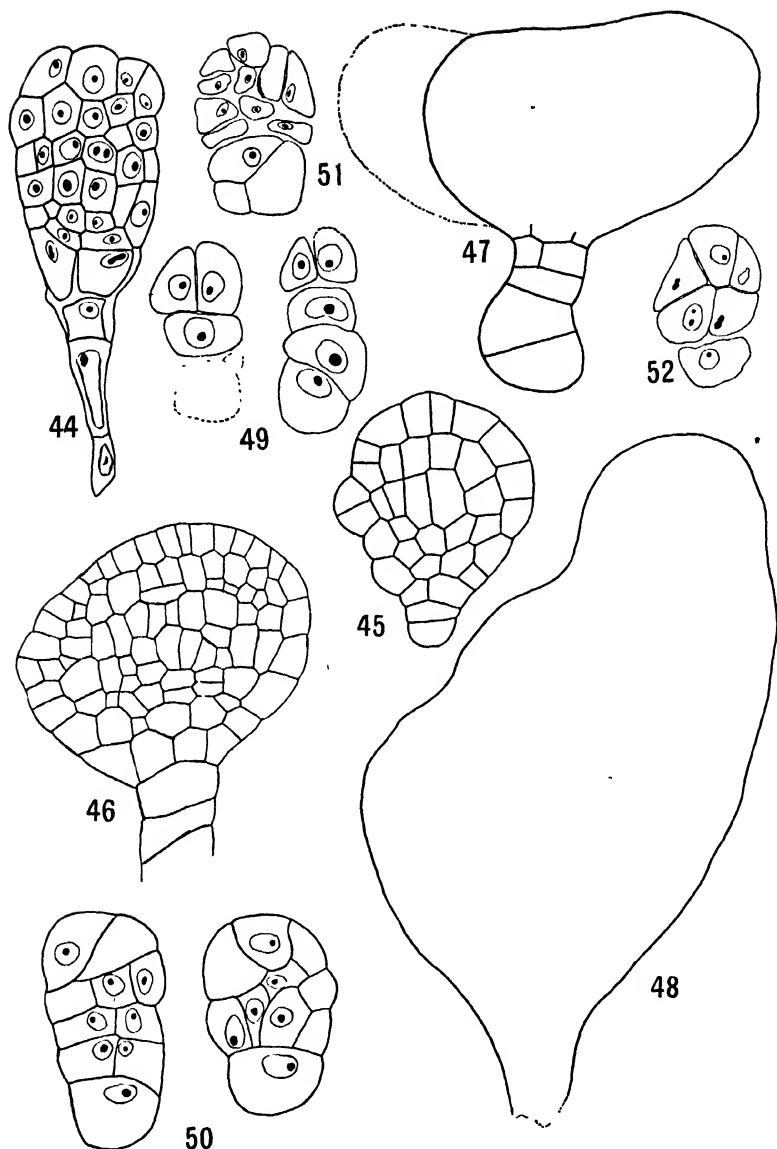
The earliest stages of the condition here described as unilaterality (or, to employ another term of similar but more significant meaning, spurious monocotyledony) are easy to detect (figs. 41-43); there seem to be two or three different types. In some cases, before the second vertical division takes place, one of the side cells is divided

more or less equally by a transverse wall (figs. 29, 31, 36, 38), and a second division occurs shortly after in the upper daughter cell. In the other cell of the 2-celled stage the normal vertical wall is sometimes formed, but a horizontal wall similar to the first in the neighboring cell is usually erected. In other types unilaterality is produced when there is an excessive number of longitudinal partitions in the apical cell and an unequal division (premature formation of the hypophysis cell?) in the basal cell (figs. 23, 34, 35). Still other cases (figs. 17, 41-43) are irregular from the beginning.

When there is order in the succession of divisions of a unilateral embryo, the result is the formation of an embryo normal in all respects save that it possesses only one normal cotyledon and the rudiments of the second (figs. 45, 46, 48). On the other hand, when there is absence of order in the divisions, an indefinite multicellular body is formed (fig. 43), which finally disintegrates. Sometimes such a structure may be found in a healthy condition in maturing seeds, but it is incapable of germination.

Fig. 43 further represents a type of embryo-like structure rather commonly encountered. Except for the large "suspensor" cell, these structures are not very different from similar embryos found in non-fasciated plants, the principal differences being that those from fasciated plants have in the aggregate a larger number of cells but lack a corresponding increase in size of the entire structure. From the large number of disorganized and disintegrating structures of this type observed, it is presumed that a lethal factor may be linked with the factor causing the formation of these embryos, thus insuring the eventual death of the monstrosities. The conspicuously large "suspensor" cell sometimes persists in an apparently healthy condition long after the remainder of the structure has completely degenerated.

The behavior of the "suspensor" deserves special notice (cf. figs. 29-31 and figs. 34-38). The only possible explanation is that, in the latter group, the primary suspensor cell failed to undergo division. In figs. 35 and 38 the diagonal wall is patently that which should have formed the concave wall in the hypophysis cell, but which failed to become concave so as to connect with the primary transverse wall at both sides.



FIGS. 44-52.—Fig. 44, columnar type of developing embryo: genesis of stem fasciation, with evidence of later split stem; fig. 45, beginning of growth (tricotyledony?) on one side of otherwise normal embryo; fig. 46, later stage of similar type; fig. 47, outline of tricotyledonous embryo; fig. 48, outline of monocotyledonous embryo (early stage in development of normal cotyledon purposely drawn to accentuate position of stem apex and aborted cotyledon); fig. 49, apogamous embryo, two successive sections; degeneration just commencing; fig. 50, same, healthy and showing no signs of disorganizing; figs. 51, 52, disorganizing apogamous embryos; $\times 450$.

TRICOTYLEDONY

This phenomenon is frequently encountered in seedlings, but the difficulties of demonstrating its appearance in the embryo with a tolerable degree of certainty are almost insurmountable. One unquestionably tricotyledonous embryo is shown in fig. 47.

DIPLOID APOGAMOUS EMBRYOS

The definite presence of these structures in one pedigree has been established. The diploidy was ascertained by counting at least twelve chromosomes in each of two mitotic figures. In structure the embryos are irregular, tending to be filamentous, and usually begin to disintegrate upon reaching about the 16-celled stage (figs. 49-52). Their origin may be attributed to the failure of the megasporocyte to undergo meiosis.

Parthenogenesis is unknown. Adventitious embryos have been observed only once, when two were found side by side on the under side (toward the embryo sac) of the only epistase found in an ovule from a fasciated plant.

Discussion

So far as can be ascertained, there is no paper in the literature on the embryology of the Dicotyledoneae which discusses the genesis of fasciation, of known cotyledonary irregularities, or of other post-germinal teratological phenomena in the embryo. The reason for this apparent deficiency is found in the fact that it is rather well agreed that these irregularities as such are not identifiable until some time beyond germination; hence it seems to have been presumed that they could not be observed in earlier stages of development.

Several opinions to account for the causes of fasciation are current, but the validity of none has been satisfactorily or conclusively demonstrated experimentally. It may be interesting to examine a few of the conjectural theories in connection with the facts here presented.

DE VRIES (20) pointed out that the first leaves appearing after the cotyledons make it possible to recognize a mutant form in *Oenothera*. This fact is also mentioned by GATES (5). Evidently the factors responsible for the creation of the new mutant begin to exercise their influence early in the life of the embryo; hence it would be interesting to study the comparative embryogeny of mutative forms of *Oeno-*

thera lamarckiana. One fact contributory to the early recognition of mutation forms is that only hypogeal seedlings exhibit fasciation. WHITE (22) thinks that (in *Nicotiana tabacum*) "the actual place in ontogeny at which the change from the normal to the abnormal took place must have been shortly after fertilization. If it had occurred later in ontogeny, the fasciated character would have appeared first as a bud-sport." As noted by WHITE, the idea that teratological variations originate more frequently under artificial conditions than in nature is not supported by the facts. He states that "unless the situation were considered carefully, one might conclude prematurely that in these plants the anomalous character is reproduced through seed," but does not know that this has been factually demonstrated. It is to be hoped that the observations recorded here may shed some light on this question, although they were not primarily directed toward this end.

In a few plants belonging to heterozygous pedigrees of *C. elegans*, fasciation and total inhibition of the inflorescence may occur simultaneously; or if buds are formed at all, they soon drop off. WHITE (22) cites GODRON to the same effect (in *Onagra biennis*). During several successive late summers this phenomenon has been common in colonies of *Epilobium angustifolium* on Fickle's Hill, east of Arcata, California. Many of these colonies are undoubtedly natural clons, and the total repression of the inflorescence in a high percentage of the ramets in each clon, whether or not fasciation is also present, points directly to the probability that the origin of the phenomenon, as well as of clonal colonies themselves, lies in the suppression in the ortet² of the faculty for the formation of sporogenous cells. The latter phenomenon is actively proceeding in many species of *Godetia*, particularly in *G. dudleyana* and *G. grandiflora*, but in these plants it leads merely to the eventual extinction of the species since they have not developed a faculty for vegetative reproduction.

No positive cytological evidence could be adduced in support of ATKINSON'S (1) statement:

The material representing the group of characters not entering into any one working combination, may then be left behind in the first suspensor cell of the embryo, or cast out into the cytoplasm, or may become subordinated. The material in the first suspensor cell, as is well known, plays no part in the formative

² For an explanation of the terms clon, ramet, and ortet, see STOUT, A.B., Jour. N. Y. Bot. Gard. 30:25-37. 1929.

processes of the new individual, since this cell is sidetracked by the basal wall formed during the first division of the zygote.

Nevertheless, it is wholly within the bounds of possibility that any one of the three suppositions expressed by ATKINSON may yet be demonstrated by cytological evidence.

Some attention was devoted to SWINGLE'S zygotaxic theory (17), but it was soon found impossible to apply this experimentally, as the very small chromosomes of *C. elegans* are morphologically so much alike that any particular pair of homologous chromosomes cannot always be distinguished from the others (fig. 6). Furthermore, as ATKINSON states: "There is . . . [no] . . . cytological evidence that the positional arrangement of the chromosomes in the cells of hybrids bears any relation to their morphological characters."

One subject closely related to that under discussion is the behavior in the embryo sac when species belonging to different subgenera of *Epilobium* are crossed. It was early discovered that whereas the cross in one direction produces normal embryos, the reciprocal cross causes the formation of an abnormal embryo or none at all. These abnormal embryos invariably degenerate, or at least are incapable of germination, after attaining the 12-celled to 20-celled stage. The cytomorphological literature on *Epilobium* crosses is too extensive to be cited here.

During this study, the possibility of predicting the appearance of teratological phenomena was demonstrated to possess a factual basis. Embryos in certain 1926 pedigrees were found to behave erratically, although the garden records indicated no irregularities of any nature. In 1927, however, the progeny from various plants of this pedigree exhibited a surprising array of aberrations, and a study of their embryology indicated that the same abnormalities would be continued during 1928. This expectation was abundantly fulfilled.

There exists considerable reason to believe that once a certain abnormality or group of concomitant abnormalities is acquired, it persists through several generations and is probably never wholly lost. There seems to be little choice in describing it as a retrogressive or an advancing evolutionary step, for it is likely to be neither: it is simply a changeable phase. As a matter of fact, there is little or nothing upon which to base a denial that so-called "abnormalities" are not in reality inseparable adjuncts to the normal existence of the

species, or, to employ a more properly descriptive term, of the linneon. Although most earlier workers considered fasciations to be in the nature of acquired characters, there is now much evidence that if fasciation is not directly a heritable character, at least a strong tendency toward the production of fasciations is carried over from one generation to the next in many species.

DE VRIES (20) summarizes the case with sufficient succinctness: Monstrosities are often considered as accidents, and rightfully so, at least as long as they are considered from the morphological point of view. Physiology of course excludes all accidentality. And in our present case it also shows that some internal hereditary quality is present, though often latent, and that the observed anomalies are to be regarded as response of this innate tendency to external conditions.

Summary

1. The genesis of various teratological phenomena is to be found during embryogeny.
2. The possible appearance of such abnormalities in progeny may be ascertained in advance.
3. They represent neither advancing nor retrogressive evolutionary steps, but in the end contribute to the changing evolutionary status of the species, for they are heritable; but fasciations caused by mechanical injury (for example, insect attacks) are not inherited.

I desire to record my great indebtedness to my Fellowship sponsors, Dr. R. A. HARPER of Columbia University, and Drs. GEORGE J. PEIRCE and L. L. BURLINGAME of Stanford University.

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A NEW SPECIES OF CUPRESSINOXYLON (GOEPPERT)
GOTHAN FROM THE JURASSIC
OF SOUTH DAKOTA

H. J. LUTZ

(WITH THIRTEEN FIGURES)

The two specimens of silicified coniferous stems here described were collected in August, 1927, by Professor WIELAND, from the *Barosaurus* horizon (Upper Como) at points two and four miles north from the town of Sturgis, South Dakota. Both were closely in place and therefore are of late Jurassic age. These fossil woods were given the writer for study in 1927. The paleobotanical laboratory at Yale University was available, and all of the section cutting, as well as the photomicrographic part of the work was done there. Ten thin sections of the type stems were cut: three transverse, three tangential, and four radial. Also, through the kindness of Professor RECORD, numerous wood specimens of living conifers were obtained from the Yale collection for comparative study.

Description

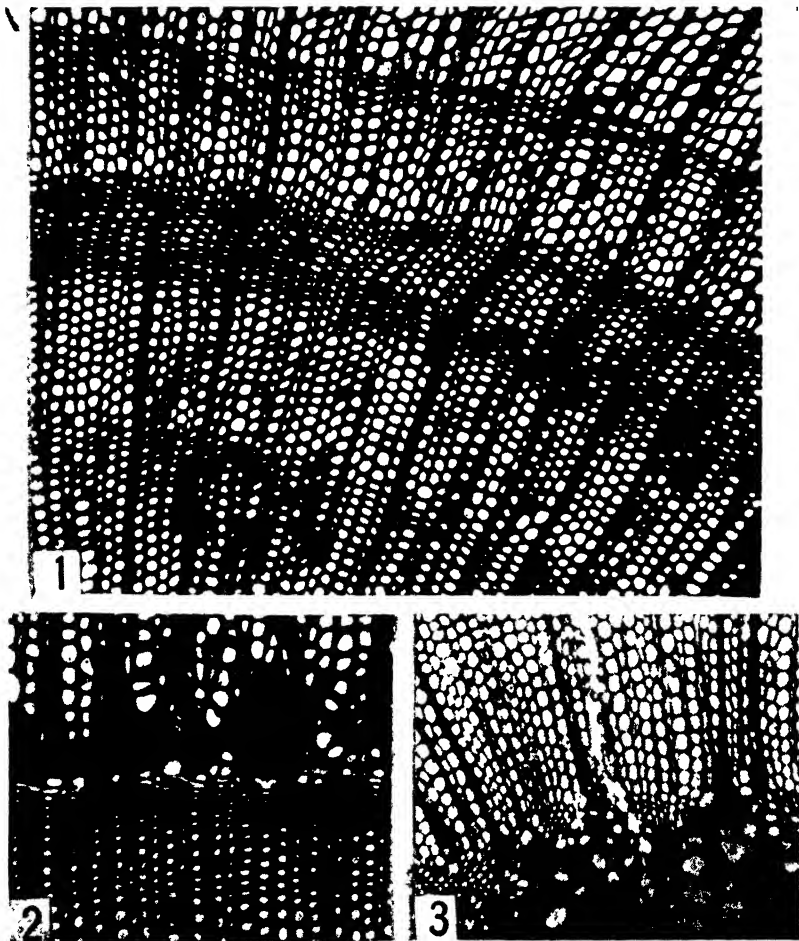
Cupressinoxylon (Goeppert) Gothan

Cupressinoxylon jurassica sp. nov.

TRACHEIDS.—The preservation is exceptionally good, silicification being of the type with left-over traces of carbon. The growth rings are notable for their complexity and show a slight tendency to be sinuate; there is also considerable ring discontinuity. The wood appears to be biannulate; that is, there can be distinguished a long growth period beginning in the early spring and running into a late summer ring, with a lesser band of fall growth ending in a sharp winter ring (figs. 1, 12). Viewed in transverse section, the spring wood tracheids appear irregularly hexagonal to nearly square in shape, and show a tendency toward tangential compression. The summer wood tracheids tend to be nearly square, although usually somewhat compressed in radial diameter; rarely they are of irregu-

larly hexagonal form. The walls are often thick, causing a reduction in size of the rounded to oval lumina.

Bordered pits on the radial walls are large, strictly uniseriate, nearly round in shape (figs. 6, 8). They may be either close set or dispersed, but occupy about the entire tracheid width. The pores are usually large and round, but occasionally are lenticular. Pits



FIGS. 1-3.—*Cupressinoxylon jurassica*: fig. 1, transverse view showing mid and late summer, followed by fall growth; winter ring and spring growth above; fig. 2, parenchymatous groups of wound tissue; fig. 3, groups of primary elements adjacent to pith; $\times 85$.

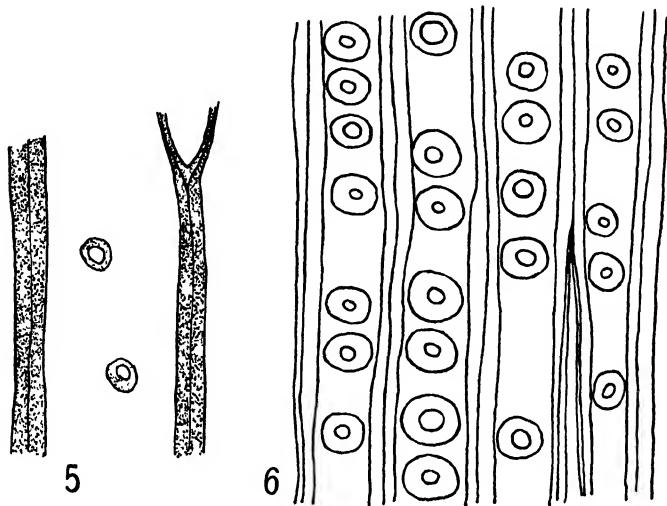
on the tangential walls occur only sparsely, and are somewhat smaller than those of the radial walls (fig. 5). They usually occupy from one-third to one-fifth of the width of the tracheid. Very faint, apparently spiral markings are distinguishable on the tangential walls of some tracheids (fig. 4). Bars of Sanio appear normal on the radial walls of the tracheids. Trabeculae are sometimes present in radial series, especially in the spring wood tracheids.



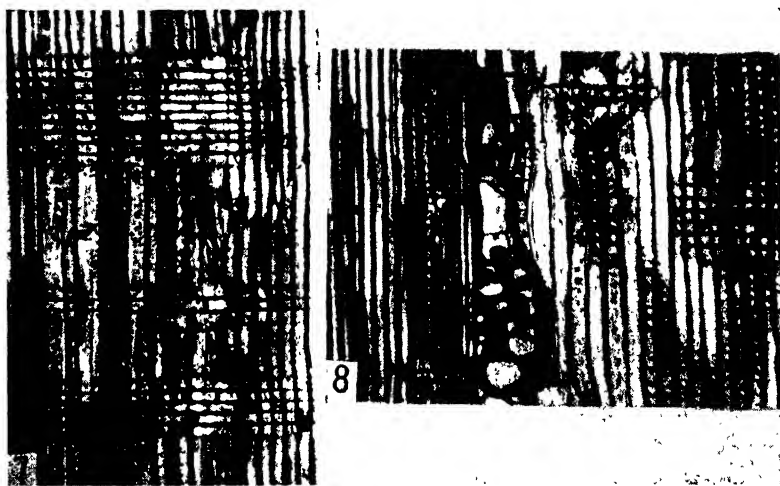
FIG. 4.—*C. jurassica*: tangential view; $\times 85$.

WOOD PARENCHYMA.—Resin cells are numerous and usually scattered, although occasionally tending toward zonation. In transverse section they appear to be about the same size as the tracheids but are more nearly square. The parenchyma cells often contain spheroid or elongate masses of black material, no doubt originally of a resinous character (fig. 10). Terminal walls are smooth and unpitted. Small bordered pits similar to those in the walls of the ray cells occur in the lateral walls of the parenchyma cells. The pores are lenticular or almost round. No resin canals are present, but there is a well defined peripheral development of wound tissue

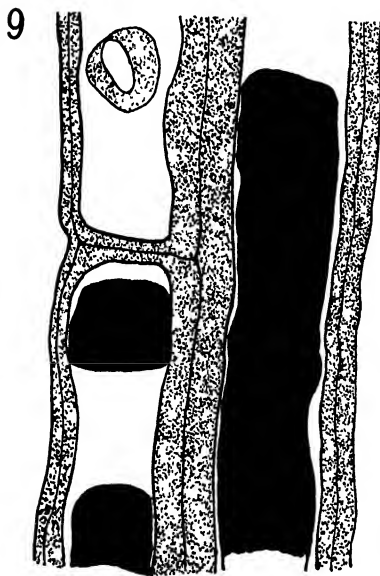
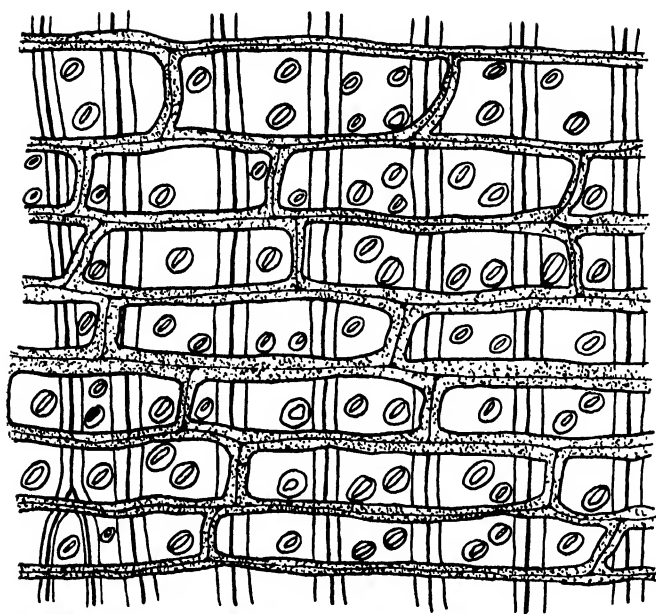
(Wundholz) in the spring wood of one growth ring (figs. 2, 8). This traumatic tissue takes the form of parenchymatous groups, and is especially developed in the region of each ray.



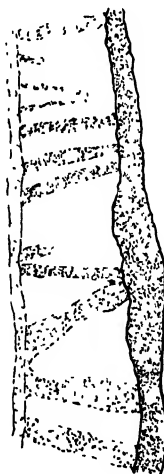
FIGS. 5, 6.—*C. jurassica*: fig. 5, pitting on tangential walls of tracheids; $\times 300$; fig. 6, pitting on radial walls of tracheids; $\times 190$.



FIGS. 7, 8.—*C. jurassica*: fig. 7, radial view; fig. 8, radial view showing wound tissue and pitting; $\times 60$.



10



11

FIGS. 9-11.—*C. jurassica*: fig. 9, radial view showing bordered pits in ray cells; $\times 200$; fig. 10, radial view of parenchyma cells; $\times 700$; fig. 11, primary element with spiral thickenings on radial wall; $\times 700$.

WOOD RAYS.—These rays are prominent, and in transverse section are four to twenty-one tracheids apart, the average distance

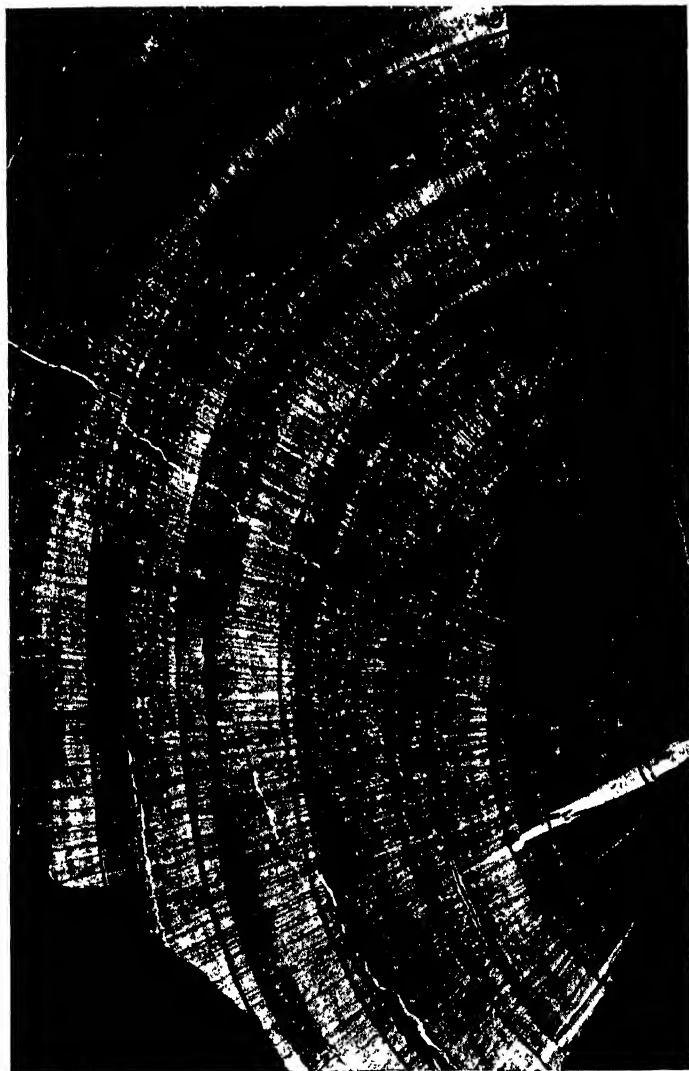


FIG. 12.—*C. jurassica*: transverse section showing combined seasonal and annual ring features. A tendency to rapid spring growth, followed by slow growth and checking during late summer dry weather, with active late fall growth and sudden winter cessation is suggested. Some of annulate shading may record the so-called "chemical growth rings" (cf. fig. 13). Eight years of growth are traversed by section. Late summer ring is dotted, and late fall ring is solid line; $\times 9.5$ (photograph by G. R. WIELAND).

being eleven (figs. 1, 3). They are predominantly uniseriate, but many are biseriate in part (fig. 4). The ray cells are often slightly

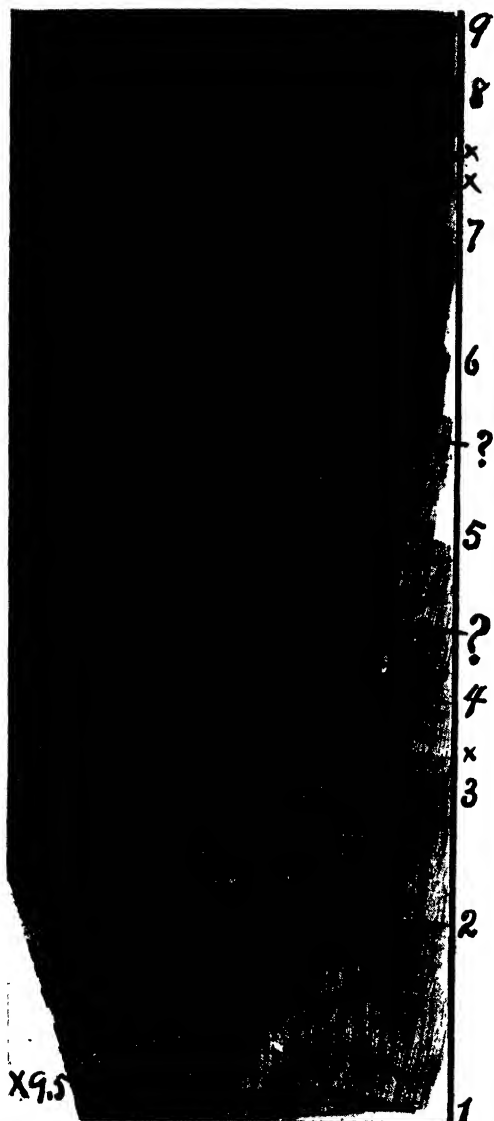


FIG. 13.—*Juniperus* sp., Cuba: transverse section showing complexity of seasonal annulation comparable to that of *Como* stem (fig. 12). As in that stem, both inequality and discontinuity appear. Such discontinuous and multiple rings are sometimes seen in rain forest conifers (*Araucaria*, Australia); $\times 9.5$ (photograph by G. R. WIELAND).

contracted near their ends, and may contain masses of black material (fig. 9). Seen in tangential section these cells are broadly oval or nearly round in shape. The tangential or terminal walls are relatively thin, unthickened, and usually curved, although not uncommonly straight (figs. 7, 9). Pitting on the lateral walls is of the "cupressoid" type, that is, the pore is relatively large, the border relatively small, and at least in the spring wood the pore is usually set in an oblique position (fig. 9). In the summer wood the pores are invariably set in a vertical position, that is, parallel to the tracheid walls. There are from one to four pits per tracheid field (Kreuzungsfeld) but the usual number is two (figs. 7, 9). The pits are nearly isodiametric, although some may be oval. The pores are usually lenticular but occasionally round. In height the

rays vary from one to twenty-four cells, the average height being seven. The number of rays per square millimeter of tangential surface varies from 9.6 to 21.7; the average is 14.8. The rays are homogeneous; fusiform rays are absent.

TABLE I
MEASUREMENTS OF WOOD ELEMENTS IN CUPRESSINOXYLON JURASSICA

MATERIAL	MINIMUM (MM.)	MAXIMUM (MM.)	AVERAGE (MM.)
<i>Growth rings</i>			
Width of rings	0 199	3 005	1.607
Width of spring wood			1 321
Width of summer wood			0.284
<i>Tracheids</i>			
(spring wood)			
Length	2 639	5 976	3 669
Radial diameter			0 0315
Tangential diameter			0 0266
Thickness of walls			0 0050
(summer wood)			
Radial diameter			0 0149
Tangential diameter			0 0259
Thickness of walls			0 0080
<i>Wood rays</i>			
Vertical diameter of cells	0 0198	0 0252	0 0227
Tangential diameter of cells	0 0180	0 0234	0 0193
Length of cells	0 0756	0 1944	0 0936
Thickness of lateral walls			0 0036
<i>Bordered pits</i>			
Diameter in ray cells			0 0078
Diameter in tracheids			0 0156
<i>Pith</i>			
Diameter			0 2000
Diameter of cells		0 103	
<i>Wood parenchyma</i>			
Length of cells			0.2466

PITH.—Cells are irregularly four- to six-sided, often containing black masses, no doubt originally of a resinous nature. The walls are thickened, probably due in part to the course of petrification (fig. 3).

PRIMARY ELEMENTS.—The protoxylem can only appear in extremely well cut sections, but the metaxylem is conspicuous. About twenty metaxylem areas surround the small pith (fig. 3). In radial section the primary elements are seen to have spiral thickenings on their walls (fig. 11).

COMPARISON OF *C. JURASSICA* WITH LIVING CONIFERS

Cupressinoxylon jurassica was compared with species of the living genera *Taxodium*, *Sequoia*, *Libocedrus*, *Juniperus*, *Thuja*, *Chamaecyparis*, and *Cupressus*. A comparative microscopic eye-piece, permitting examination of two sections at the same time, was used in making most of the comparisons.

In *Juniperus* and *Libocedrus* the tangential walls of the ray cells are almost invariably thickened, while in *Cupressinoxylon jurassica* these walls are relatively thin. Further, in *Libocedrus* the pits on the lateral walls of the ray cells are simple or with an inconspicuous border. In *Taxodium* and *Sequoia* the bordered pits in the radial walls of the tracheids are multiseriate; in *C. jurassica* they are strictly uniseriate. Further, in *Taxodium* the cross walls of the parenchyma cells are greatly thickened and the pits in the radial walls of the tracheids are characteristically crowded, flattened, and often irregularly arranged(RECORD 21).

Cupressinoxylon jurassica was also compared with descriptions (sections for microscopic study not being at hand) of *Podocarpus*, *Thujopsis*, *Cryptomeria*, *Saxegothaea*, *Fitzroya*, *Actinostrobus*, *Widdringtonia*, *Callitris*, and *Arthrotaxis* as given by PRILL (19) and PENHALLOW (16). A review of the differences between these genera and *C. jurassica* will not be given, but it should be stated that in all cases some structural variation is indicated. So far as the living Cupressineae are concerned, *C. jurassica* most closely resembles *Cupressus* and *Chamaecyparis*.

In general the tangential ray cell walls of *Cupressinoxylon jurassica* are curved, agreeing with *Cupressus* (15). PENHALLOW (16) states that in *Chamaecyparis lawsoniana*, *C. pisifera*, and *C. obtusa* the tangential walls of the ray cells are thin and entire, while in *Cupressus macrocarpa*, *C. arizonica*, *C. macnabiana*, *C. goveniana*, *Chamaecyparis thyoides*, and *C. nootkatensis* the walls are somewhat thickened. The tangential ray cell walls in *C. jurassica* being relatively thin and entire, agree in this respect with the former group.

In both *Cupressus goveniana* and *C. macnabiana* the number of rays per square millimeter of tangential surface is much greater than in *Cupressinoxylon jurassica*. In the two former species the rays are much lower as a rule, and the pitting on the lateral walls

of the rays smaller in size than in *C. jurassica*, but of the same general type. In *Chamaecyparis nootkatensis* the number of rays per square millimeter is higher than in *C. jurassica*, but the difference is not so marked as in species of *Cupressus*. The ray cell pitting of *Chamaecyparis* is similar to that in *C. jurassica*, but the pits of *Chamaecyparis* are somewhat smaller.

The occasional occurrence of ray tracheids in certain species of both *Cupressus* and *Chamaecyparis* reported by JONES (7), PENHALLOW (15, 16), and JEFFREY (6) is not observed in *C. jurassica*. PENHALLOW (16) states that in *Chamaecyparis pisifera* and *Cupressus macrocarpa* pits in the radial walls of the tracheids are in one row or in pairs; the remaining species, *Chamaecyparis obtusa*, *C. lawsoniana*, *C. nootkatensis*, *C. thyoides*, *Cupressus macnabiana*, *C. arizonica*, and *C. goveniana* having strictly uniseriate pits. *C. jurassica* resembles the latter group in this respect.

JONES (7) figures a transverse section of *Cupressus macrocarpa*, showing peripheral groups of parenchymatous tissue which appear very similar to the development of wound tissue in *C. jurassica*. It appears, therefore, that *C. jurassica* bears considerable resemblance to the living genera *Cupressus* and *Chamaecyparis*. This appears in size and arrangement of the elements, as well as in more specific characters such as ray cell pitting.

One interesting difference between *C. jurassica* and living species of *Cupressus* and *Chamaecyparis* from Arizona, California, and southwestern Alaska is the greater density of the summer wood of the latter genera. Not only is it of greater density, but the boundary between the spring and summer wood of each growth ring is much sharper. A brief annual period of rest is indicated in the Como stems by the rather sharp transition from one annual growth ring to the next. A wet spring, dry late summer, warm wet late fall, and short dry, even frosty winter, would explain the *C. jurassica* rings. It seems reasonable that the late Jurassic climate was characterized by a less marked seasonal variation than prevails in the California coast region of North America at present.

Cupressus, with ten or twelve species, is confined to the Pacific Coast region of North America and Mexico in the New World, and to southwestern Europe, southwestern Asia, the Himalayas, and China in the Old World (PILGER 17, SARGENT 22). *Chamaecyparis*,

with six species, is confined to the Atlantic and Pacific Coast regions of North America, and to Japan and Formosa (17, 22).

COMPARISON OF *C. JURASSICA* WITH FOSSIL CONIFEROUS WOODS

Cupressinoxylon is briefly described by KRÄUSEL (11). A large number of fossil coniferous stems have been referred to this genus. KNOWLTON (8) lists twenty-seven species of *Cupress(in)oxylon*, together with one species each of *Cupressus* and *Cupressites* from North America. KRÄUSEL (9) records over 170 specimens, which were variously referred to *Cupressinoxylon*, *Cupressoxylon*, *Cupressus*, and *Cupressites*. In many cases the generic names *Cupressinoxylon* and *Cupressoxylon* have been used synonymously in describing the same species, many of the specimens were not determined as to species, and several of them were question marked. Some of the determinations were based on poorly preserved or scanty material, so that it is not surprising that the identifications cannot be authenticated. KRÄUSEL (9) limits the number of forms which may be referred with reasonable certainty to *Cupressinoxylon* to seventeen species, as follows:

Species	Age	Occurrence
1. <i>Cupressinoxylon wellingtonioides</i>	Miocene	Silesia
2. <i>C. cupressoides</i>	Miocene	Silesia
3. <i>C. gothani</i>	Miocene	Silesia
4. <i>C. patagonicum</i>	Oligocene	Patagonia
5. <i>C. sylvestre</i>	Tertiary	Russia
6. <i>C. severzowi</i>	Tertiary, Cretaceous (Greensand)	Russia
7. <i>C. sanguineum</i>	?	Russia
8. <i>C. sp.</i> Kräusel	Miocene	Silesia
9. <i>C. sp.</i> Gothan	Tertiary	Spitzbergen
10. <i>C. sp.</i> Prill	Miocene	Silesia
11. <i>Cupressoxylon podocarpoides</i> . . .	Tertiary (also Cretaceous?)	Japan
12. <i>Cupressus pritchardi</i>	Eocene	Ireland
13. <i>Rhizocupressinoxylon liasinum</i> . . .	Liassic	Orne, France
14. <i>Libocedrus sabiniana</i>	Tertiary	Greenland
15. <i>Sequoia canadensis</i>	Miocene	Canada
16. <i>S. magnifica</i>	Tertiary	Yellowstone Park, North America
17. <i>Biota orientalis</i> var. <i>miocenica</i>	Miocene	Silesia

STOPES (24) lists four species of *Cupressinoxylon* as follows:

1. <i>Cupressinoxylon vectense</i>	Lower Greensand	Isle of Wight
2. <i>C. luccombense</i>	Lower Greensand	Isle of Wight
3. <i>C. cryptomerioides</i>	Kentish Rag	Maidstone
4. <i>C. hortii</i>	Lower Greensand	Woburn

Cupressinoxylon jurassica was compared with these species and found to differ in every case. The chief differences noted are as follows.

1. *Cupressinoxylon wellingtonioides*, according to the description given by PRILL and KRÄUSEL (20), differs from *C. jurassica* chiefly in the greater height of its rays (a maximum of thirty-five cells being noted) and the commonly biseriate character of the pits on the radial walls of the tracheids. Further, the rays of *C. wellingtonioides* appear to be largely uniseriate and small vertical resin canals appear in the wound tissue.

2. *Cupressinoxylon cupressoides* differs from *C. jurassica* chiefly in that its parenchyma cells occur in two to three tangential bands. The pitting on the radial walls of the tracheids is biseriate in part, and pits on the tangential walls of the tracheids are numerous. There may be as many as two pits per tracheid field on the lateral walls of the ray cells. The rays are usually low, the highest having twenty-three cells; seldom are they biseriate (9, 10).

3. *Cupressinoxylon gothani* (9, 10) seems to be similar to *C. jurassica* in some respects. The chief differences lie in the somewhat higher maximum heights of the rays of *C. jurassica* and in the greater tendency for the tangential walls of the ray cells to be curved in *C. jurassica*. KRÄUSEL (10) shows a commonly biseriate condition of pitting. In this respect also it differs from *C. jurassica*.

4. *Cupressinoxylon patagonicum* is stated by KRÄUSEL (9) to be a coniferous wood without resin canals, belonging to *Cupressinoxylon*, but which cannot be determined more closely.

5. *Cupressinoxylon sylvestre* differs from *C. jurassica* in several particulars. FELIX (2) quotes MERCKLIN (14) as describing a concentric grouping of the parenchyma cells in *C. sylvestre*. MERCKLIN stated that the rays varied from two to twelve cells in height.

6, 7. *Cupressinoxylon severzowi* cannot be distinguished from *C. sanguineum* according to KRÄUSEL (9). The latter species is a coniferous wood without resin canals, that cannot be determined

more closely. Possibly it may belong to *Cupressinoxylon*. *C. jurassica* cannot be carefully compared with either of these species.

8-10. *Cupressinoxylon* sp. Kräusel was not determinable as to species (9, 10), due to poor preservation of the material. The maximum height of the rays is given as twelve cells. *C. sp.* Gothan and *C. sp.* Prill both represent specimens which in consequence of their poor preservation cannot be determined as to species (9).

11. *Cupressoxylon podocarpoides* is stated (9) to be a coniferous wood without resin canals, which possibly belongs to *Cupressinoxylon*, but which cannot be determined more closely because of insufficient description.

12. *Cupressus pritchardi* is, in the words of KRÄUSEL (9), too briefly described for certain determination. GARDNER (3) bases his description on vegetative characters and cones, and MACLOSKIE (13) figures only a small radial view of the wood. In MACLOSKIE's figure the tangential walls of the ray cells are not shown, and the pitting on the lateral walls appears to consist of large simple pits, or if bordered, the pore has been omitted.

13. *Rhizocupressinoxylon liasinum* is considered to be the oldest true *Cupressinoxylon* by GOTHAN (4). KRÄUSEL (9) states that the arrangement of the pitting of the tracheids in this species is not entirely clear. LIGNIER (12) states that there are thirty-six rays per square millimeter of tangential surface. The average number per square millimeter in *Cupressinoxylon jurassica* is about fifteen. The rays of *R. liasinum* are considerably lower than in *C. jurassica*, and the number of pits per tracheid field on the lateral walls of the ray cells is from three to five, which is somewhat higher than in *C. jurassica*. In addition, the pitting on the lateral walls of the tracheids is biseriate in *R. liasinum*.

14. *Libocedrus sabiniana* cannot be determined as to species with certainty, since the structure of the medullary rays is not clear (9).

15. *Sequoia canadensis* cannot be carefully compared with *C. jurassica* since it is impossible to make a closer determination than to say that the specimen belongs to *Cupressinoxylon* (9).

16. *S. magnifica*, according to PENHALLOW (16), has biseriate pitting, at least in part, on the radial walls of the tracheids. Due to poor preservation of the specimen, KRÄUSEL (9) states that it

cannot be determined more closely than as belonging to *Cupressinoxylon*.

17. *Biota orientalis* var. *miocenica*, according to KRÄUSEL (9), is a typical cupressinen wood whose peculiar "intercellulars" point with certainty to *Biota*. In *C. jurassica* the "intercellulars," or what only appear to be such, are normal.

Of the four species of *Cupressinoxylon* listed by STOPES (24) from the English Lower Greensand, all differ specifically from *C. jurassica*, although *C. vectense* is nearest, and has the complex growth rings, but with much lower wood rays. *C. cryptomerioides* has slight growth rings and quite low wood rays; *C. luccombense* has low wood rays and variant ray pitting; *C. hortii* has wood rays of extraordinary abundance, height, and width, being perhaps separated generically from other *Cupressinoxylon*.

RELATIVE AGE OF SPECIES HAVING CUPRESSINEAN AFFINITIES

In view of the age of *Cupressinoxylon jurassica*, it is of interest to note the ideas held as to the age of species having cupressinean affinities. SEWARD (23) states: "It is undoubtedly true that in the later Jurassic and Lower Cretaceous floras, conifers agreeing generally in habit and in the possession of appressed imbricate leaves with such genera as *Cupressus*, *Chamaecyparis*, and *Thuya* were among the most characteristic types." JEFFREY (5) states that "all evidence goes to show that the Taxodineae and Cupressineae did not exist before the very end of the Cretaceous or more probably before the beginning of the Tertiary." COULTER and CHAMBERLAIN (1) state that although *Cupressus*-like twigs and cones have been described from the Jurassic, there is no reliable evidence of the tribe earlier than the Upper Cretaceous. POTONÉ and GOTHAN (18) state that *Cupressinoxyla*, *Glyptostroboxyla*, and *Taxodioxyla* are common in the Tertiary as "Braunkohlenhölzer."

Summary

A new species, *Cupressinoxylon jurassica*, from the late Jurassic of South Dakota, is described and figured. This species is the oldest that has been certainly referred to *Cupressinoxylon*. The determination is based on microscopic study of ten thin sections and compari-

son with fossil and living coniferous woods. A strictly modern conifer type, in all respects, is indicated.

The writer wishes to acknowledge the aid of Dr. G. R. WIELAND in this study, particularly in the interpretation of the seasonal annulation of the Como stems.

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CYTOLOGICAL STUDIES ON THE BETULACEAE

IV. BETULA, CARPINUS, OSTRYA, OSTRYOPSIS

ROBERT H. WOODWORTH

(WITH THIRTY-FOUR FIGURES)

It has been pointed out in the previous papers of this series (WOODWORTH 4, 5) that polymorphism in the Betulaceae is due, in part at least, to the readiness with which the species cross in nature. It has also been noted that certain irregularities of the reduction division leading to polycary, polyspory, sterile pollen, parthenogenesis, apogamy, polyembryony, polyploidy, and polymorphism suggest a heterozygous origin for plants showing any of these characters (WOODWORTH 6).

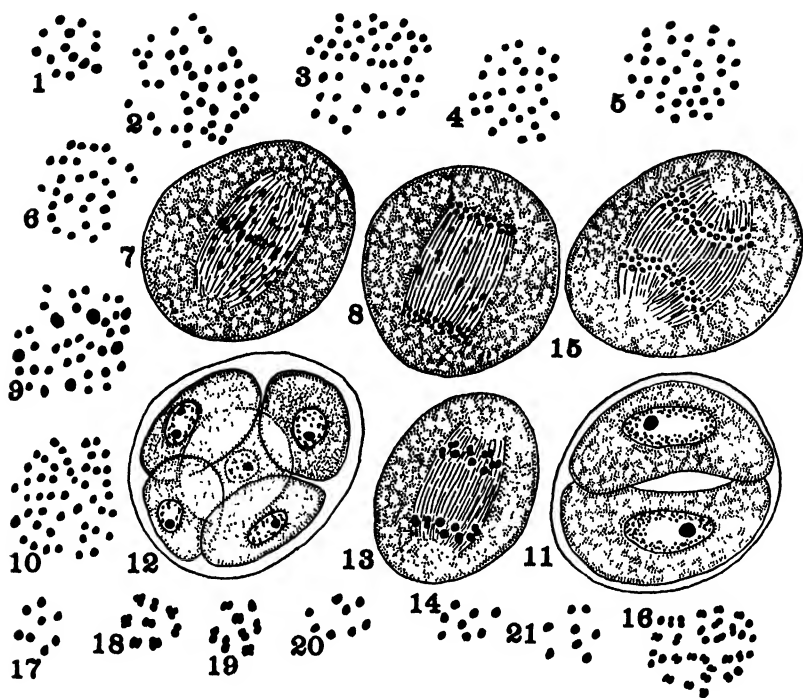
Materials and methods are explained in the first paper of this group (4). Professor C. O. ROSENDAHL has very kindly sent me collections from the birches growing in Minnesota.

BETULA (CONTINUED)

Betula utilis var. *prattii* Burk, X-14 (meiosis normal).—Fig. 1 shows a metaphase plate of the first division with fourteen chromosomes.

B. lutea Michx. f., X-42 (meiosis normal).—The material reported on previously by the writer (4) was collected in Massachusetts. The material here dealt with is from Minnesota. The meiosis is identical and shows the species in both regions to be hexaploid. Fig. 2 shows a metaphase plate of the first division with forty-two chromosomes. It was interesting to find that this is so, because *B. lutea* is one of the parents in \times *B. purpurea* treated in this paper.

B. papyrifera Marsh. and its varieties are most interesting because of their extreme polymorphism. True *B. papyrifera* and the variety *cordifolia* have been found to have thirty-five (pentaploid) and twenty-eight (tetraploid) respectively as the reduced number of chromosomes (4). Three other varieties are reported here. Such work on the morphology of the chromosome groups may help in untangling their polymorphism.



FIGS. 1-21.—Fig. 1, *Betula utilis* var. *prattii* Burk., metaphase plate of first division showing 14 chromosomes. Fig. 2, *B. lutea* Michx. from Minnesota, metaphase plate of first division showing 42 chromosomes. Fig. 3, *B. papyrifera* var. *occidentalis* Sarg., heterotypic metaphase plate showing 42 chromosomes. Fig. 4, *B. papyrifera* var. *subcordata* Sarg., heterotypic metaphase plate showing 28 chromosomes. Fig. 5, *B. papyrifera* var. *kenaica* Henry, heterotypic metaphase plate showing 35 chromosomes. Fig. 6, *B. pumila* var. *glandulifera* Regel, heterotypic metaphase plate showing 28 chromosomes. Figs. 7-12, \times *B. purpurea* Schneid. (*B. pumila* var. *glandulifera* \times *B. lutea*): fig. 7, metaphase of first division showing many univalent chromosomes; fig. 8, anaphase of first division showing lagging univalent chromosomes; fig. 9, heterotypic metaphase plate showing 36 vari-sized chromosomes; fig. 10, heterotypic metaphase plate showing 47 chromosomes; fig. 11, diad of diploid pollen grains; fig. 12, polyspory, five pollen grains rather than normal tetrad. Figs. 13, 14, *Carpinus betulus* L.: fig. 13, anaphase of first division showing 8 chromosomes; fig. 14, heterotypic metaphase plate showing 8 chromosomes. Figs. 15, 16, *C. betulus* var. *fastigiata* Nichols: fig. 15, anaphase of first division showing 32 chromosomes; fig. 16, heterotypic metaphase plate showing 32 chromosomes. Fig. 17, *C. caroliniana* Walt., heterotypic metaphase plate showing 8 chromosomes. Fig. 18, *C. laxiflora* Bl., diakinesis showing 8 pairs of chromosomes, several split for second division. Fig. 19, *C. turezaninovi* Hance., diakinesis showing 8 pairs of chromosomes, one pair already split for second division. Fig. 20, *C. orientalis* Mill., heterotypic metaphase plate showing 8 chromosomes. Fig. 21, *C. japonica* Bl., heterotypic metaphase plate showing 8 chromosomes.

B. papyrifera var. *occidentalis* Sarg., X-42 (meiosis normal).—This variety differs from the true species and the variety just noted in being hexaploid. Fig. 3 shows the metaphase plate of the first division with forty-two chromosomes.

B. papyrifera var. *subcordata* Sarg., X-28 (meiosis normal).—Fig. 4 shows twenty-eight chromosomes in the heterotypic metaphase plate. This variety is tetraploid.

B. papyrifera var. *kenaica* Henry, X-35 (meiosis normal).—This is the only variety of the four studied which is pentaploid like *B. papyrifera*. Fig. 5 is the heterotypic metaphase plate with thirty-five chromosomes.

B. pumila var. *glandulifera* Regel, X-28 (meiosis normal).—Fig. 6 shows the metaphase plate of the first division with twenty-eight chromosomes. When noting the decided correlation of results of ROSENDAHL'S (3) taxonomic work and the writer's cytological work on $\times B. sandbergi$ (*B. papyrifera* \times *pumila* var. *glandulifera*) (4), the *B. pumila* var. *glandulifera* parent had not been available for investigation. *B. pumila* was found to have twenty-eight pairs of chromosomes, and the chromosome counts of *B. sandbergi* indicated that the variety must have the same number as the true species. This is now seen to be correct. The material used was sent from Minnesota by Professor ROSENDAHL. This variety is one of the parents of the hybrid birch discussed just below.

$\times B. purpusii$ Schneid. (*B. lutea* \times *pumila* var. *glandulifera*), 2X-70; X about 45 (meiosis very abnormal).—The cytological study of this hybrid has proved to be a distinct correlation and conclusion to the taxonomic studies of ROSENDAHL (3), whose observations extended over several years. His drawings of the leaves, catkins, cone scales, and seeds show *B. purpusii* to possess characters which are intermediate, in every way, between those of the swamp birch and the yellow birch. He points out that there are but three species of *Betula* in Minnesota, where he found this hybrid. These are *B. papyrifera*, *B. pumila* var. *glandulifera*, and *B. lutea*. Hybrids between the first two of these have already been described taxonomically by ROSENDAHL (3) and cytologically by the writer (4). In the taxonomic work, photomicrographs of pollen of the three species show it in each case to be 100 per cent perfect. The two hybrids show 33 and 45 per cent of the pollen defective.

The *lutea* parent of this hybrid has forty-two as the reduced number of chromosomes. The *pumila* var. *glandulifera* parent has twenty-eight. Metaphase plates of sporophytic cells in the hybrid contain about seventy chromosomes. The latter are so small and so close together that an accurate count is almost impossible.

The nuclei in diakinesis contain so many chromatic elements that they are most difficult to study. It appears that a majority of the chromosomes from the *glandulifera* parent find synaptic mates and appear during the first division as bivalent chromosomes. Those which do not pair and the extra fourteen or more from the *lutea* parent lag on the spindle. Fig. 7 shows the metaphase of the first division. Pollen mother cells in the phase viewed from one of the poles show from thirty-six (fig. 9) to forty-seven (fig. 10) chromosomes. In cases where there are less than forty-two, some of the *lutea* chromosomes may have paired among themselves, or several chromosomes may have fused into one mass, as fig. 9 suggests (WOODWORTH 5). In the anaphase of the first division the chromosomes which did not pair are very tardy in their movements on the spindle (fig. 8). The second division is also typified by such random movements of the chromosomes. Frequently a number of these laggards are not included in the nuclei at the spindle poles and they form extra pollen grains (fig. 12).

At times the tardiness is so marked that all the chromosomes on the spindle are included in one nucleus, which then forms a broad spindle and produces a diad of diploid pollen grains (fig. 11). This is the semiheterotypic division (ROSENBERG 2) which was also found in *B. sandbergi* and other species (WOODWORTH 4). This production of polyploid gametes is now becoming better understood (KARPECHENKO 1). It is an excellent explanation of hybridity as an important cause of polyploidy.

Many of the anthers show cytomyxis and chromatolysis to a marked degree. This was found to be the case in other species which were probably of hybrid origin (WOODWORTH 4). Occasionally a long string of pollen mother cells is seen to be fused together. From one-third to one-half of the pollen is morphologically sterile.

All species of *Betula*, *Alnus*, and *Corylus* studied by the writer (4, 5) have fourteen as the fundamental number of chromosomes.

The other three genera, *Carpinus*, *Ostrya*, and *Ostryopsis*, have eight as the fundamental number.

CARPINUS (TOURN.) L.

Carpinus betulus L. X-8 (meiosis normal).—Figs. 13 and 14 represent the anaphase and metaphase plate of the first division. Both show eight chromosomes.

C. betulus var. *fastigiata* Nichols, X-32 (meiosis normal).—This variety is an octoploid form. The pollen mother cells are accordingly several times larger than those of *C. betulus*. Figs. 15 and 16 show the anaphase and metaphase plate of the first division with thirty-two chromosomes.

C. caroliniana Walt., X-8 (meiosis normal).—Fig. 17 shows eight chromosomes in the heterotypic metaphase plate.

C. laxiflora Bl., X-8 (meiosis normal).—Fig. 18 represents the diakinesis with eight pairs of chromosomes, some of which are split for the second division.

C. turczaninowii Hance, X-8 (meiosis normal).—Fig. 19 shows the diakinesis with eight pairs of chromosomes, one pair already split for the second division.

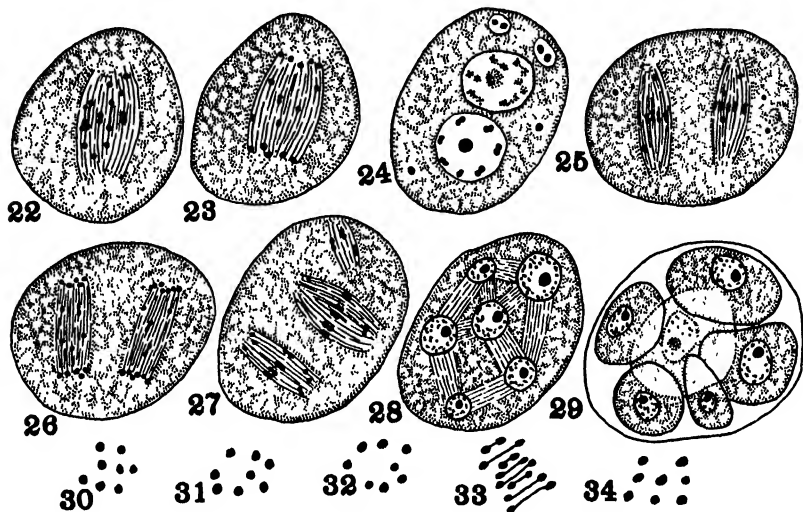
C. orientalis Mill., X-8 (meiosis normal).—Fig. 20 shows eight chromosomes in the heterotypic metaphase plate.

C. japonica Bl., X-8 (meiosis normal).—Fig. 21 is the heterotypic metaphase with eight chromosomes.

C. cordata Bl., X-8 (meiosis very abnormal).—The diakinesis is an easy stage to study because there are so few chromosomes. Characteristically several of the chromosomes do not pair. These appear on the spindle as univalents, as in the early anaphase shown in fig. 22. In the late anaphase they lag on the spindle (fig. 23). Some of the laggards do not enter the interkinetic nuclei but form micronuclei (fig. 24). The homeotypic division is also very irregular. Fig. 25 shows the metaphase with lagging and extruded chromosomes. The anaphase shows these laggards (fig. 26). Fig. 27 portrays an anomalous homeotypic division with three abnormal spindles, the extra spindle apparently having been formed by extruded chromosomes. These abnormalities lead to the formation of polycaric mother cells. Fig. 28 shows such a cell with six nuclei rather than the normal tet-

rad. Polyspory results (fig. 29). Large numbers of the pollen grains degenerate.

In studying the cytology of the several species comprising a genus, it is interesting to find most of the species passing through the meio-



FIGS. 22-34.—Figs. 22-29, *Carpinus cordata* Bl.: fig. 22, early anaphase of first division showing unpaired chromosomes; fig. 23, late anaphase of first division showing lagging univalent chromosomes; fig. 24, interkinesis: nuclei have 6 and 7 chromosomes respectively, the other 3, already split, reposing in cytoplasm; fig. 25, homeotypic metaphase showing lagging and extruded chromosomes; fig. 26, homeotypic anaphase showing lagging chromosomes; fig. 27, anomalous homeotypic division with three abnormal spindles; fig. 28, polycaric mother cell showing 6 nuclei; fig. 29, polyspory, 6 pollen grains instead of normal tetrad. Fig. 30, *Ostrya virginiana* K. Koch., heterotypic metaphase plate showing 8 chromosomes. Fig. 31, *O. virginiana* var. *glandulosa* Sarg., heterotypic metaphase plate showing 8 chromosomes. Fig. 32, *O. japonica* Sarg., heterotypic metaphase plate showing 8 chromosomes. Fig. 33, *O. carpinifolia* Scop., early anaphase of first division showing 8 chromosomes. Fig. 34, *Ostryopsis davidiana* Dcne., heterotypic metaphase plate showing 8 chromosomes.

tic division in a wholly normal manner, and one of the species possessing the meiotic irregularities just discussed. Since the materials from all of the plants were collected at the same time, and treated throughout the technical procedure in exactly the same manner, it is obvious that there is an innate unbalance in the cells themselves. The results of these abnormalities, polycary and polyspory, show

that the phenomenon is a natural one having nothing to do with the fixative. In fact, these irregularities can be seen in living material.

Carpinus cordata (that is, the plants growing in the Arnold Arboretum) shows the meiosis which is well known to be typical of hybrid plants, and is therefore put under suspicion of having arisen by heterozygosis. There is a very interesting particular concerning the bract which subtends the nutlets. In all other species of *Carpinus* this bract is flat. In *Ostrya* species the nutlet is inclosed in a tubular involucre open at the top. The bract of *Carpinus cordata* is not flat as in the other species of the genus, but is inflexed at the base covering the nutlet. This advances the possibility that *C. cordata* may be a bigeneric hybrid between *Carpinus* and *Ostrya*.

OSTRYA (MICH.) SCOP.

Ostrya virginiana K. Koch., X-8 (meiosis normal).—Fig. 30 shows the heterotypic metaphase plate with eight chromosomes.

O. virginiana var. *glandulosa* Sarg., X-8 (meiosis normal).—Fig. 31 shows the heterotypic metaphase plate with eight chromosomes.

O. japonica Sarg., X-8 (meiosis normal).—Fig. 32 shows the heterotypic metaphase plate with eight chromosomes.

O. carpinifolia Scop., X-8 (meiosis normal).—Fig. 33 shows the early anaphase of the first division with the eight pairs of chromosomes just separating.

OSTRYOPSIS DCNE.

Ostryopsis davidiana Dcne., X-8 (meiosis normal).—Fig. 34 shows the heterotypic metaphase plate with eight chromosomes.

Summary

1. *Betula lutea* from Minnesota is hexaploid as is the New England plant.

2. *B. papyrifera* and its varieties have interesting chromosome numbers. *B. papyrifera* has the haploid number of thirty-five; *B. papyrifera* var. *cordifolia* has twenty-eight; *B. papyrifera* var. *subcordata* has twenty-eight; *B. papyrifera* var. *kenaica* has thirty-five; *B. papyrifera* var. *occidentalis* has forty-two. It is hoped that this will help to explain the polymorphism in the group.

3. *B. pumila* var. *glandulifera* has twenty-eight pairs of chromosomes.

4. $\times B. purpusii$ (*B. lutea* \times *pumila* var. *glandulifera*) presents another complete document, taxonomically and cytologically, of natural hybridization.

5. *Carpinus*, *Ostrya*, and *Ostryopsis* species have eight as the fundamental number of chromosomes.

6. *Carpinus betulus* var. *fastigiata* is octoploid with thirty-two chromosomes as the reduced number.

7. *Carpinus cordata* shows all the cytological characteristics of a hybrid and is accordingly suspected of having a heterozygotic ancestry. It may be a bigeneric hybrid.

8. The existence of polyploidy and irregular meioses in polymorphic plants, as here reported, furnishes more evidence for the theory that multiplication of species has come about to a considerable extent by hybridization.

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BRIEFER ARTICLES

IS FASCIATED A FREQUENTLY MUTATING CHARACTER?

The fasciation of *Pharbitis nil*, the Japanese morning glory, is manifested by certain polymeric genes. The writer¹ has already described three genes, fasciated-1, fasciated-2, and fasciated-3. Owing to the reduplicated nature of the genes on the one hand, and the possible existence of modifiers on the other, the segregating proportions of fasciated to normal are often complicated and deviate from the expectations. Some genotypically fasciated species have uncertain manifestations of fasciation; in other words, the fasciated pedigrees frequently give some normal atavists among their progeny, sometimes containing even a majority of normals. HAGIWARA² regarded them as produced by gene mutation. On selfing 5 fasciated plants obtained in hybrid progeny, he got 119 individuals containing 41 atavists.³ He also called attention to the occurrence of reverse mutations from normal to fasciated. This possibility, however, is based on rather unconvincing facts. Of course, our fasciated species originated spontaneously from normal ancestors, and this origin would surely be due to mutation. This mutation, however, seems to be very rare. As to the occurrence of normal atavists among fasciated sisters, YAMAGUCHI⁴ also reported as follows:

Was zunächst das oftmalige Auftreten der Atavisten (Rückmutanten) in den verschiedenen Linien mit fasziertem Stengel anbetrifft, so scheinen mir darunter zwei Kategorien unterschieden werden zu sollen. Die Atavisten, die nach der Selbstbefruchtung ausschliesslich fasziierte Nachkommen geben, wie ich bisher beweisen konnte, sollten der einen Kategorie angehören, während sie

¹ IMAI, Y., Experiments with a pear-leaved and fasciated strain of the Japanese morning glory. Jour. Genetics 18:275-314. 1927.

² HAGIWARA, T., Genetic studies of the fasciation in morning glories. Bot. Mag. Tokyo 40:281-294. 1926 (Japanese).

³ Most of them were noted as having non-pear leaves among otherwise pear leaves. To the writer, such non-pear and non-fasciated plants would have suggested contamination rather than double mutation.

⁴ YAMAGUCHI, Y., Notiz über die Vererbung der Fasziation bei *Pharbitis nil*. Bot. Mag. Tokyo 40:535-537. 1926.

sich bei der anderen wieder in die ihr ähnlichen normalen und faszierten zu spalten pflegen. Die erste Kategorie könnte man etwa falsche Atavisten (Pseudo-Rückmutanten bzw.-Rückmutation) nennen, während es sich bei der zweiten um die echten Atavisten, d.h. um die Rückmutation des Gens handelt.

In other words, YAMAGUCHI accepted HAGIWARA's view that fasciated is a frequently mutating character, but at the same time he recognized its fluctuation resulting in a normal character. YAMAGUCHI's paper being in the nature of a short note, he did not give numerical data supporting his view.

The writer, however, concluded that these normal atavists are due merely to fluctuation in manifestation of the character fasciated. His evidence was furnished by the hybrid progeny derived from a cross between non-fasciated no. 326 and fasciated no. A5. An F_3 examination of this cross covers progenies of 183 F_2 plants, of which 35 are pear and non-fasciated and 5 are pear and fasciated. Of these 35 pear and non-fasciated F_2 plants, two proved, on examination of their offspring, to be really pear and fasciated; that is, they were false normals. Combining these results with those of properly fasciated plants, the data contain 65 individuals, of which 6 are normal atavists. When the previous paper was written, these were all the data available to support the writer's opinion that atavists are due merely to the fluctuating manifestation of the character fasciated. In the successive years two further generations of fasciated F_3 plants have been raised to prove this view and settle the problem.

On selfing two fasciated F_3 plants of pedigree no. 169, of which the F_2 mother plant was a false normal (atavist), the writer obtained F_4 offspring consisting of 16 fasciated and 10 normal plants and 33 fasciated and 9 normal plants respectively. From the former pedigree an F_5 generation was reared. The F_5 offspring of 16 fasciated F_4 consisted of 162 fasciated and 42 normal plants, and those of 9 normal F_4 contained 66 fasciated and 17 normal plants. The proportions of normal atavists in these pedigrees are 21 per cent in the former and 20 per cent in the latter, giving practically the same results. These data collectively do not furnish anything for the mutation view, but give indisputable evidence for the fluctuation view. The genes for fasciated, therefore, are of constant state, and the frequent appearance of normal atavists among fasciated sisters is due to a fluctuating representation. These atavists are of a temporary form. Returning to HAGIWARA's data, they do not give any positive evidence for his mutation view. For YAMAGUCHI's view we cannot argue beyond his statement, because he did not present numerical data.

The proportion of false normals among fasciated sisters is very variable, owing partly to various genotypes of the fasciated complex and partly to the fluctuating manifestation of the fasciated character. One of the writer's fasciated strains, no. A5, is very fixed for the representation of the characteristic and contains almost no atavists. YAMAGUCHI⁵ stated that his fasciated strains were also highly fixed for this character. In his extensive cultivation⁶ of these pedigrees he obtained only 1 per cent of normal atavists among some 1200 or 1300. Another of the writer's fasciated strains gives about 15 per cent of false normals, although the proportion varies to some extent in the different cultures. The fasciated pedigrees obtained in the hybrid progeny are generally more unstable, through the interference of certain modifiers. The commercial strains, however, are generally of high fixation for the fasciated characteristic, because some intense selection would have been made before their registration.—Y. IMAI, *Botanical Institute, Agricultural College, Tokyo Imperial University*.

⁵ YAMAGUCHI, Y., On the inheritance of fasciation in *Pharbitis nil*. Proc. Jap. Assn. Adv. Sci. 2: 264-273. 1926 (Japanese).

⁶ Owing to the defective sexual organs fasciated strains generally give few seeds.

CURRENT LITERATURE

BOOK REVIEWS

Oedogoniaceae

The long awaited monograph of the Oedogoniaceae¹ by TIFFANY has appeared, and it justifies the hope that a great university, with three members of its botanical staff specializing in algae, would bring out a work of the first order. Foreign universities have long recognized the desirability of comprehensive monographs, written by specialists in the various groups; but in America such monographs have been more or less limited to the seed plants. Exceptions, like the monograph on *Isoetes* by PFIEFFER, are not numerous. Monographs like COLLIN's *Green algae of North America*, while useful and stimulating, are too local.

For the past 30 years the principal monograph of the Oedogoniaceae has been HIRN's *Monographie und Iconographie der Oedogoniaceen*. This work has become hard to secure, and, during the 30 years since its publication, many new species have been described and many have been emended. TIFFANY's study of American and foreign forms, extending over a period of 15 years, has fitted him for undertaking an up-to-date monograph.

After a presentation of the well known facts of cell structure and life history, there is an interesting account of periodicity and distribution. In the north central United States the maximum of sexual reproduction in *Oedogonium* is reached in May and in July, in both annual and perennial species, with a second maximum, not nearly so vigorous, in October. In the same area 54 per cent of the species are found in permanent ponds, 23 per cent in lakes, 15 per cent in temporary ponds, 6 per cent in streams, and 2 per cent in stream oxbows. *Bulbochaete* shows a similar behavior. In *Bulbochaete* 42 species, two varieties, and one form are dioecious, nanandrous; six species, two varieties, and one form are monoecious; there are no dioecious macrandrous species. In *Oedocladium* three of the four species are monoecious and one dioecious. All are nanandrous. In *Oedogonium* 61 species, 15 varieties, and 8 forms are monoecious; 56 species, 25 varieties, and 11 forms are dioecious, macrandrous; and 77 species, 36 varieties, and 19 forms are dioecious, nanandrous.

It is hard enough to define a species, but varieties and forms are still more indefinite. As TIFFANY uses the terms, an alga which occurs year after year in

¹ TIFFANY, L. H., *The Oedogoniaceae*, a monograph including all the known species of the genera *Bulbochaete*, *Oedocladium*, and *Oedogonium*. 8vo. pp. 253. pls. 64. Published by the author. Columbus, Ohio. 1930.

the same habitat with a known species, but differs in one or more morphological characters, but is evidently closely related to the type, is a *variety*. If an alga differs from the description of a species, but has been seen only once or twice, one cannot know whether a variation is constant or is merely an ecological variation. The term *form* is used for such cases.

The keys are in English and are particularly definite; probably the average botanist who has had a course in the morphology of algae could use them. This statement cannot be made in regard to many keys in thallophytes where a genus has such a large number of species as is found in *Bulbochaete* and *Oedogonium*.

A monograph on the Zygnemaceae is in preparation, and it is hoped that others will follow.—C. J. CHAMBERLAIN.

Statistical methods for research workers

A third edition of FISHER's monograph² follows closely the publication of the second edition.³ There is little difference between the second edition and the third. The chief change is made near the end of the final chapter on the principles of statistical estimation, where section 57 is followed by two new subsections on fragmentary data, and on the amount of information, design, and precision. These sections occupy 10 pages, and illustrate some wider applications of the method of maximum likelihood, and the quantitative evaluation of information. In the citations of sources used for data and methods at the end of the book, the author has removed his own contributions to a separate list, in which he includes practically all of his papers, whether pertinent or not.

It is doubtful whether a third edition was necessary at this time. There should be some compelling reason for multiplying editions of books, however good they may be.—C. A. SHULL.

² FISHER, R. A., Statistical methods for research workers. 8vo. pp. xvi+283. Edinburgh and London: Oliver and Boyd. 1930.

³ BOT. GAZ. 87:670-671. 1929.

THE BOTANICAL GAZETTE

October 1930

POTASH SHALE AS A SOURCE OF POTASSIUM FOR GROWING PLANTS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 405

HARRY C. HEATH

(WITH NINE FIGURES)

Introduction

Numerous attempts have been made to manufacture profitably soluble potassium fertilizer from potash rocks. While these rocks were our least source of supply during the emergency caused by the late war, they contain our greatest quantity of potassium because of their wide distribution. Ross (25) says that while the United States has the greatest phosphate mines in the world, it has twenty times more potash. Most of it, however, is confined in these difficultly soluble rocks of low grade, none of them over 10 per cent potash. The five principal forms of rock potash are orthoclase feldspar, greensand, alunite, leucite, and shale. They all belong to the group of silicate rocks except alunite, which is a hydrous potassium aluminum sulphate. GARDINER (11) states that 128 patents on processes for treating silicate rock for the extraction of potash were issued between 1904 and 1917. The European deposits being richer and very soluble, American manufacturers cannot compete with the foreign product, except when the rocks can be used in other forms of manufacture and the potash obtained as a by-product.

Many experiments have been made using potash rocks pulverized and applied directly to the soil as a source of potassium for plants.

There are some advantages in using mineral fertilizers in this form. While they require heavier initial application, they are cheaper and do not leach out so readily. In the case of potassium these less soluble rock fertilizers are never harmful, while heavy applications of the soluble forms sometimes prove injurious because of their bad effects on soil structure (13), because of impurities they contain as by-products of other forms of manufacturing (22), and because they may prevent the formation of nodules on the roots of legumes (20).

Most of the earlier experiments with orthoclase feldspar seemed to give favorable results, as for example, those of MAGNUS, AITKEN, NILSON, and BALLENTINE, all quoted by DE TURK (9), who likewise obtained promising results. However, most of the later investigators reported unfavorably. Among these are HARTWELL and PEMBER (15), BROOKS and GASKILL (7), VANATTA (34), and PRIANISCHNIKOV (24).

Certain greensands or glauconites of the Atlantic Coast have long been used locally without pulverizing. TRUE and GEISE (31), SKEEN (28), and BLAIR (4) found them useful as sources of potassium. Much of the greensand is so low (1.6–5.8 per cent) in potassium content, however, that it requires very heavy application.

Comparatively few experiments have been carried out with potash shales, but most of them have shown considerable potassium available for growing plants. STEWART (29) tried a Nevada shale and compared it with leucite, alunite, and kainit, finding that in potassium-deficient peat this shale produced better growth than any other potash carrier tested. PARR and others at the Illinois Experiment Station (23) obtained a better growth of corn with 2.68 tons of Union County shale than with five-sixths of a ton of kainit per acre. On the other hand, Dixon County shales were of little benefit. This difference in fertilizer value of the two shales is attributed to a difference in the form in which the potash is held in each. Sixty-two per cent of the potassium in the Union County shale was made soluble by treatment with concentrated sulphuric acid, while the acid had little effect upon the solubility of the potassium in the Dixon County shale. They think that the potassium made soluble in the Union County shale was in the glauconitic form. SCHMITT (26) secured a good response to the pulverized Decorah Shale of Minne-

sota. Sixty per cent of the potassium in this shale was made soluble by the concentrated sulphuric acid treatment.

The largest outcrop of potash shales known in this country is near Cartersville, Georgia. The State Geological Survey (19) reports it as approximately half a mile wide and 15 miles long. It has been drilled 400 feet without reaching bottom. STOCKETT (30) names the three great potential sources of potash in the United States as the leucite hills of Wyoming, the greensands of the Atlantic Coast, and the potash shales of Georgia. Possibly there may be added to these the deep potash deposits of western Texas which have been reported from the logs of oil wells. The Cartersville shale contains 6.6-8 per cent potassium, and has been used in this investigation.

Methods

In all, twelve kinds of plants were grown, in 676 containers. Some were grown in quartz sand and others in potash-deficient soils, of

TABLE I
POTASSIUM CONTENT OF SHALE AND OF SOILS, AND
LIME REQUIREMENT OF SOILS

MATERIAL	POTASSIUM PERCENTAGE	LIME REQUIREMENT FOR ALFALFA (TONS PER ACRE)
Cartersville shale	7.9	
Peat from University of Wisconsin	0.25	2
Peat from Coddington Farm.	0.51	2
Plainfield sand	0.70	2
Hazel Crest sand	1.74	4

which four types were used. The sample of shale, kindly furnished by Mr. J. T. NORRIS of Cartersville, was of light olive drab color, rather soft, and easily pulverized. Table I shows the potassium content of the shale and the soils, together with the lime requirements of the soils as indicated by the Truog test.

QUARTZ SAND

The quartz sand used was no. 3 $\frac{1}{2}$, manufactured by the Wausau Quartz Company and claimed by them to contain practically no plant nutrients. The shale was ground in a mill and in a mortar

until it passed a 100-mesh sieve. Six-inch earthenware pots were washed and dipped for 2 minutes into melted paraffin. Approximately 1415 cc. of quartz sand weighing 1892 gm., and the individual portions of shale were mixed well and poured together into each pot. The shale was added on the basis of tons per acre, assuming a 15 cm. depth of soil weighs 824,155 kg. per acre. The plants grown in quartz sand were white sweet clover, tomatoes, corn, squash, and cotton. In all except the squash, seven rows of pots were used and each row contained eight pots. Each of the rows from no. 2 to no. 6 were watered with Shive's solution $R_5C_2(27)$, modified by using an equivalent of PO_4 in $Ca(H_2PO_4)_2$ in place of KH_2PO_4 . The solutions were made up in tap water, which the analysis of the Board of Health of the City of Chicago showed to contain only 0.9 parts per million of potassium. Row no. 1 was watered with tap water and row no. 7 with Shive's solution complete. The amount of potassium in the tap water was thought too low to affect the results. The solutions had to be used abundantly because of the heat in the greenhouse, so they were used one-fifth strength. The squash plants grew four weeks, the corn and tomatoes six weeks, and the cotton and sweet clover ten weeks.

PEATY SOILS

Peaty soils were used because they are often deficient in potassium, and because it was thought that the large amount of organic matter with its naturally occurring acids and its attendant increase in carbon dioxide and bacterial action in nitrification might increase the solubility of the potassium in the shale, as was indicated by the work of AMES and BOLTZ (1). The first peat used was a virgin soil from the University of Wisconsin marsh, furnished by the State Department of Soils through the courtesy of Dr. GEIB. It will hereafter be designated as the University of Wisconsin peat. The second peat was obtained from the Coddington State Experiment Farm in Portage County, Wisconsin, kindly furnished by Dr. E. TRUOG. It will be called the Coddington peat. These peats are chocolate brown in color when dry but brownish black when wet. They contain many partially decayed roots and stems, and were put through a 6-mm. mesh sieve to remove the coarsest materials. After this each was thoroughly mixed. Corn and clover were grown in the

University of Wisconsin peat in 6-inch tinned-fruit cans coated inside with melted paraffin and having five 6-mm. holes in the bottom of each. Tomato plants followed the corn in the same cans of soil without addition of fertilizer. Buckwheat and oats were grown in the Coddington peat in paraffined 6-inch pots. The corn and clover were grown in quintuplicate in large south windows from January to May, with the rows of the series running at right angles to the plane of the windows. The tomatoes were grown in triplicate in the same situation. The buckwheat and oats were grown out of doors (in Chicago) on a bench during July and August.

SANDY SOILS

Sandy soils were used for comparison with the peats and quartz sand. Plainfield sand, a soil deficient in potassium, was obtained from the University of Wisconsin. The second sandy soil was collected from Hazel Crest, Illinois, about 20 miles south of Chicago. It was taken from a field lying next to the west side of the Illinois Central Railroad right-of-way, just south of 171st Street. It was once part of the lake bed of the old Calumet Stage of Lake Chicago, and lies about a quarter of a mile inside of the old Calumet Beach. The first test with buckwheat seemed to indicate that it was deficient in potassium, but later results showed that it was not potassium-deficient for corn. It is a dark brown sandy loam with a high acidity, and since it has not been named by a soil survey, it will be designated as Hazel Crest sand.

Alfalfa and winter wheat were grown in the Plainfield sand, the former in triplicate in paraffin-coated tin cans, and the winter wheat in single 3-gallon stone crocks with a 12-mm. hole in the bottom of each. The wheat was grown from December 1 to May 3 and the alfalfa from January to May, both in south windows.

Alfalfa and medium red clover were grown in triplicate in the Hazel Crest sand in the same sort of 6-inch cans and in south windows from January to May. Corn was also grown in the Hazel Crest sand, but out of doors during July and August in 1-gallon glazed crocks with a 12-mm. hole in each. The crocks were set on boards in the bottom of a hotbed without artificial heat and without glass except during rain. The fertilizers were added on a basis simi-

lar to that used in the quartz sand. As indicated in the tables, the University of Wisconsin peat was limed at a rate of 2.5 tons per acre with Missouri limestone fine enough to pass a 60-mesh sieve. This limestone was found to neutralize 97.3 per cent as much dilute nitric acid as did the chemically pure precipitated CaCO_3 used in the outdoor experiments. One-half of each row of oats and buckwheat grown in the Coddington peat was treated with the equivalent of 2 tons per acre of chemically pure precipitated CaCO_3 . The Plainfield sand was limed for alfalfa at the rate of 2 tons per acre of Missouri limestone, as was also one-half of the crocks of the same soil in which was grown winter wheat. Most of the Hazel Crest soil was given 4 tons of limestone to the acre. All of the plants except those grown in the quartz sand were watered with distilled water.

USE OF GYPSUM AND LIMESTONE WITH SHALE

It has been claimed by some gypsum producers and some soil workers (2, 8, 35) that gypsum increases the availability of insoluble forms of soil potassium; therefore combinations of gypsum and potash shale were used. In the quartz sand and in the University of Wisconsin peat, some of the rows received equal quantities of pulverized shale and chemically pure CaSO_4 mixed with the soil at the same time. In all the other experiments, one row of pots was treated with shale that had been mixed thoroughly with about 20 per cent of its own weight of CaSO_4 and roasted at red heat for 30 minutes. In the Coddington peat and in the two sandy soils, one row of pots with each kind of plant received the shale roasted with 15 or 20 per cent of its weight of CaCO_3 . In the sandy soils, two additional rows of pots were treated with the shale which had been autoclaved at 20 pounds steam pressure for 30 minutes with gypsum and CaCO_3 respectively. To learn whether any of the soils were deficient in sulphur, and therefore responded to the gypsum, two extra control rows were grown. One was usually numbered 1b, and it was untreated the same as the control except that it received gypsum. The other row received KCl and gypsum.

Chemical analyses

In order to determine whether the potash shale increased the intake of potassium in the plants grown on it, the dry tops of five sets

of plants were analyzed for potassium. A sodium cobalti-nitrite method was used, which was originally perfected by BOWSER (6) and modified by KOCH. It consisted in oxidizing the tissue by the wet method, using concentrated sulphuric acid with a few drops of fuming nitric acid. The acid was then evaporated to dryness over a low flame, and the potassium salt with traces of other sulphates heated to redness to drive off any trace of acids or of ammonium sulphate. The residue was taken up in about 2 cc. of ammonium-free water, acidified with 1 cc. of glacial acetic acid, and precipitated with a strong solution of sodium cobalti-nitrite. It was evaporated on a steam bath to a paste to insure complete precipitation. The paste was stirred with approximately 25 cc. cold water and the precipitate filtered out on an asbestos pad in a Gooch crucible. The pad and precipitate were transferred to a beaker, stirred with 25 cc. water, then acidified with 5 cc. of approximately 50 per cent sulphuric acid. This was boiled with an excess of N/20 potassium permanganate solution and decolorized with N/20 oxalic acid. Enough of the same potassium permanganate was used to bring the color back to a faint pink. The difference between the amount of permanganate used and the amount of oxalic acid gave the amount of permanganate required to oxidize the potassium-sodium cobalti-nitrite precipitated. One cc. of N/20 KMnO_4 is equivalent to 0.0003595 gm. potassium. When tested in comparison with the Lindo-Gladding method on pure solutions and on plant tissues, the cobalti-nitrite method was found to run approximately 1 per cent higher, but to be more constant in the presence of the impurities in the plants, as was found by DODD (10). HAMID obtained better results with the cobalti-nitrite method than with the perchloric acid method (14). The slightly higher results, being constant, do not affect the comparison of the potassium content in the plants.

Nine of the thirteen rows of corn grown in Hazel Crest sand were analyzed for nitrogen and carbohydrates, as well as for potassium. This corn tissue was preserved in approximately 80 per cent alcohol to which was added a little CaCO_3 . To separate the soluble from the insoluble material, the alcohol was drained off and the tissue extracted with a Wylie or a Landsiedl extractor for 4 hours. The tissue was dried in a 100° oven and ground in a mortar to pass a 40-mesh sieve, and extracted five times with hot water. After this it was

extracted in approximately 75 per cent alcohol for 12 hours. Young plant tissues like corn contain so little ether-soluble materials that it was thought unnecessary to carry out the usual ether extraction. The preserving alcohol and the water and the alcoholic extracts were poured together and made up to volume. Total solids were determined in the usual way. The extract and tissue were next analyzed for total nitrogen by the Gunning method, modified to include nitrate nitrogen (3). Each was then analyzed for nitrate plus ammonia nitrogen by the Official Zinc-iron Method (3) and recorded as such in the table. The percentage of ammonia plus nitrate nitrogen was subtracted from the total nitrogen in the extract and the difference recorded as soluble organic nitrogen. These analyses were repeated with the tissue and the difference recorded as insoluble organic nitrogen.

Reducing sugars were estimated by the Official Munson and Walker general method (3) and with the volumetric permanganate method with the solution standardized against sodium oxalate. Non-reducing sugars were inverted for 7 minutes at 70°, making the total time of heating 10 minutes, with 5 cc. concentrated HCl (sp. gr. 1.19) in 75 cc. solution. The amount of non-reducing sugars was found by difference between the percentages of reducing sugars before and after hydrolysis. Polysaccharides were estimated in the extract and in the tissue as directed in the Official Methods for starch (3) and recorded as invert sugars. No clearing agent was used, for trials with and without a clearing agent gave such slight differences that they were thought to be within the experimental error. Similar possibilities are suggested by LOOMIS (18), and similar results were obtained with and without clearing agents by MORRIS and WELTON (21).

Results

QUARTZ SAND

Table II and figs. 1, 2, and 3 show the results obtained with quartz sand. The growth responses are indicated in the table in percentages of gain over the control. The variation in the responses of different kinds of plants to potash fertilizer is very striking. With the complete nutrient solution, there was an increased growth of

11.1 per cent in sweet clover and of 119.8 per cent in corn. The increases with potash shale varied from 14.8 to 62.1 per cent, omitting the squash which was tested with only 1.5 tons per acre. The ratios between the gains in potash shale and the gains in complete Shive's solution are just as variable, but this ratio is largest when the gains are smallest. With the corn, the shale caused about 40 per cent as great a response as was produced by the complete Shive's solution; with tomatoes, 65 per cent; cotton, 120 per cent; and sweet

TABLE II
PLANTS GROWN IN QUARTZ SAND; INCREASED GROWTH GIVEN
IN PERCENTAGE GAIN OVER CONTROL

No	SHIVE'S SOLUTION USED	TONS OF SHALE	CORN*		TOMATOES*		SQUASH*		COT- TON*	SWEET CLOVER*
			Green	Dry	Green	Dry	Green	Dry	Green	Green
2 . . .	No K	0 0	control							
3 . . .	No K	0 5	7 5	18 3	33 6	Lost			9 8	14 8
4 . . .	No K	1 5	28 6	43 2	22 1	Lost	12 5	11 8	5 7	14 2
5 . . .	No K	3 0	29 8	43 2	14 0	Lost			19 5	12 0
6 . . .	No K	6 0	47 4	62 1	61 7	Lost			17 1	1 9
7	With K	0 0	119 8	97 4	90 8	Lost	61 8	64 5	15 5	11 1

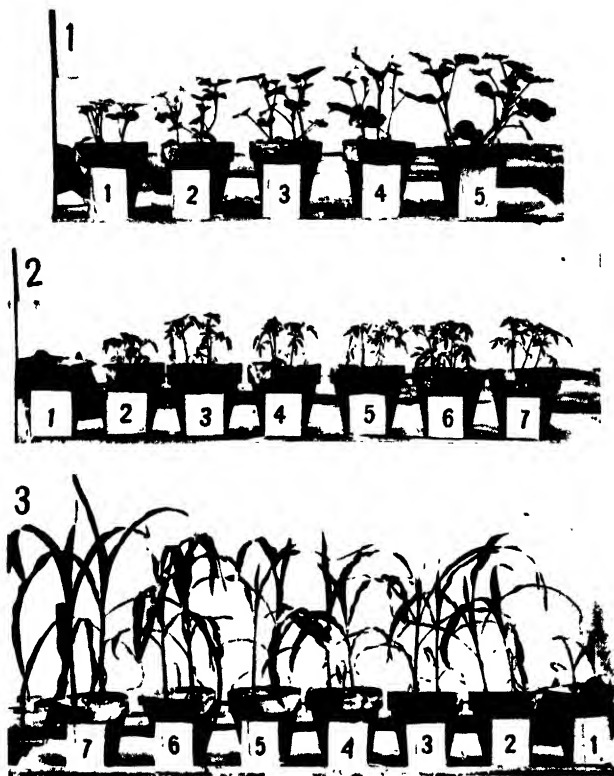
* The squash grew 4 weeks, the tomatoes and corn 6 weeks, and the cotton and sweet clover 10 weeks.

clover, 150 per cent. Some of this variation may have been due to the excessive heat in the greenhouse accompanied with unequal ventilation.

UNIVERSITY OF WISCONSIN PEAT

Figs. 4, 5, and 6 and tables III and X show results obtained with the University of Wisconsin peat supplemented with shale and KCl. The corn and the clover in table III were grown on virgin peat, but the tomato plants of table X were grown, without further fertilization, on the same soil and in the same vessels that had grown the corn. The corn made rather consistent gains in weight and in the percentage of potassium when grown with potash shale. The responses in clover were almost proportional to the amounts of shale used. Gypsum caused enormous increases in the clover in rows no. 1b and no. 6b compared with companion rows which received similar treatment but no gypsum. This indicates that the University of Wisconsin peat is deficient in sulphur for clover. The control rows no.

rb (table III), in both clover and corn, contain a lower percentage of potassium than the companion rows without gypsum. With clover, the deficiency of sulphur in the peat and the addition of gypsum caused a great increase in growth in spite of the lack of potassium. The increase in dry weight with the limited intake of potassium decreased the percentage of potassium. In no. 4b also, of the clover,



FIGS. 1-3

the potassium content was lower than in the control. This was grown on raw shale. Later in this paper it is shown that roasting shale increases the availability of the potassium that it contains. This accounts in part for the lower percentage of potassium in plants grown with raw shale. The equally good growth of clover on roasted and unroasted shale may be due to a slight amount of sulphur in the

shale. Analyses by the Georgia State Department of Geology show that the shale contains 0.1 per cent sulphur.

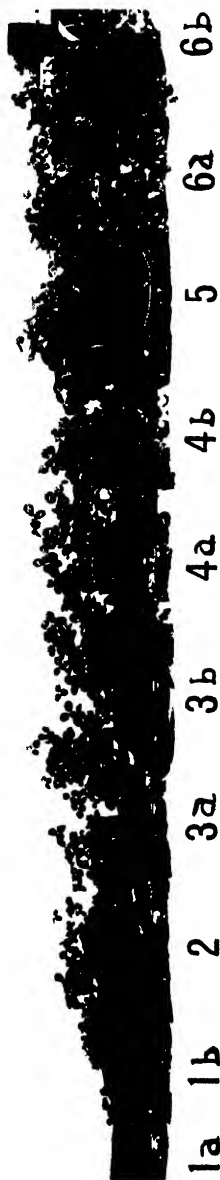


FIG. 4

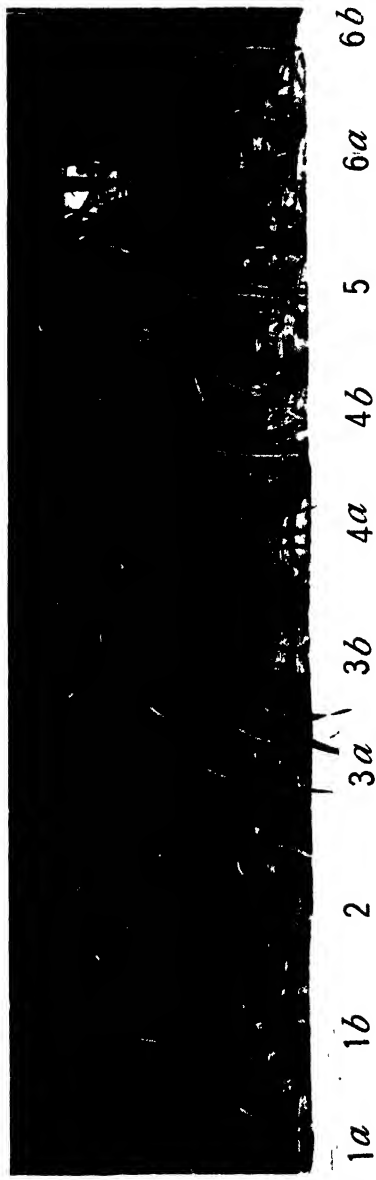


FIG. 5

Rows no. 3a and 3b test the effect of the presence of gypsum on the availability of the potassium in the shale. There is little effect seen either in growth or percentage of potassium in the tissues of the



FIG. 6

corn, but there is a doubling of the growth and a 25 per cent increase in the percentage of potassium in the clover. This gain in growth in the clover was probably due to the furnishing of sulphur, but the rise in potassium content may be attributed to an increased availability of the potassium, due to the CaSO_4 .

In rows no. 4a and 4b a test is made of the relative availability of the potassium in roasted and in raw shale. Very little difference is manifest in the growth of the clover, but the corn exhibits about 60 per cent greater increase of growth, and the potassium percentage is about 50 per cent higher in both kinds of plants, indicating a much greater availability of the potassium in the roasted shale.

CODDINGTON PEAT

The results with buckwheat and oats grown in Coddington peat are shown in table IV and in figs. 7 and 8. Each row of four pots in this experiment contained two pots treated with 2 tons of chemically pure precipitated CaCO_3 per acre and two pots untreated. The results with

CaCO_3 are shown in lines in which the numbers are followed by "L." No gains over the control are recorded for oats in pots treated with both CaCO_3 and shale or KCl, but five out of seven groups receiving some form of potassium without lime carbonate show increases in weight. The gains with shale vary from 0.4 to 38.2 per cent with an average of 20.6 per cent. All five applications of shale to buckwheat, except one, show benefit in dry weight,

TABLE III

PLANTS GROWN IN PEAT FROM UNIVERSITY OF WISCONSIN MARSH; INCREASED GROWTH GIVEN AS PERCENTAGE GAIN OVER CONTROL

No	TONS OF SHALE	CaSO_4 TONS	CORN*		MEDIUM RED CLOVER†			
			Growth		K (per- centage in shoots)	Growth		K (per- centage in shoots)
			Green	Dry		Green	Dry	
1a	o o control	o o	. . .		1 97	.	.	1 59
1b	o o	1 3	33 6	38 8	1 30	133 2	188 5	1 05
2	o 43 roasted	o o	20 0	20 9	1 92	38 2	39 5	1 62
3a	1 3 roasted	o o	25.9	17 9	2 53	102 6	111 4	1 82
3b.	1 3 roasted	1 3	24 0	21 9	2 35	209 5	212 4	2 28
4a	2 6 roasted	o o	28 3	26 3	3 81†	243 7	231 9	2 07
4b	2 6 raw	o o	7 0	16 4	2 07	246 4	253 8	1 36
5 . .	5 2 roasted	o o	63 3	63 7	3 07	529 0	526 2	2 60
6a. . .	218 o lb. KCl	o o	71 7	68 9	5 98	402 1	380.9	4 60
6b.	218 o lb. KCl	1 3	70 5	57 3	6 29	527 2	524 8	4 45

* The corn grew 11 weeks and the clover 23 weeks.

† The peat in which the clover grew was treated with 2.5 tons of Missouri limestone per acre.

‡ Probably too high; the tissue was disintegrated by mold.

and even this exception shows benefit in green weight. The percentages of increased growth range from 0 to 30.9, with an average of 17. In all the shale-treated rows the gains were about 50 per cent better in the unlimed part of each row. Thus lime injured this peat for both oats and buckwheat. In rows 5 and 7 may be compared the effects of roasted and raw shale. In the absence of lime carbonate, roasted shale produced a gain of 30 per cent as compared with no gain on raw shale, and a slight increase in the percentage of potassium. In the presence of CaCO_3 , however, roasting nearly trebled the increase in weight and raised the percentage of potassium about half. Roasting the shale with gypsum seemed to depress the growth of buckwheat in the unlimed pots in row 4, and to have no effect in

the limed pots, but it increased the percentage of potassium nearly half in both limed and unlimed pots. Roasting the shale alone, and with gypsum, had no effect on the growth of oats. Numbers 4 and 5

TABLE IV

PLANTS GROWN IN PEAT FROM CODDINGTON EXPERIMENT FARM; GROWTH SHOWN AS PERCENTAGES OF GAIN OVER CONTROL; K GIVEN IN PERCENTAGE OF TISSUE

No.	TONS PER ACRE			BUCKWHEAT			OATS		
				Growth		Potas- sium intake	Growth		Potas- sium intake
	Shale	Gypsum	CaCO ₃	Green	Dry		Green	Dry	
1	0 0	0 0	0 0	Control		0 94	Control		1 41*
1L	0 0	0 0	2 0	1 6	- 0 4†	lost	-28 6	-28 5	0 99*
1b	0 0	0 5	0 0	- 0 42	0 9	1 16	7 9		
1bL	0 0	0 5	2 0	- 0 42	- 1 1	0 91	-21 5		
3	1 3r†	0 0	0 0	13 7	10 5	1 13	38 2	38 5	
3L	1 3r	0 0	2 0	- 0 03	4 2	0 96	- 4 9	-11 1	
4	2 6R1	0 5	0 0	24 4	19 3	2 83	25 6		2 45*
4L	2 6R1	0 5	2 0	19 1	16 3	2 36	-21 3		2 31*
5	2 6Ro	0 0	0 0	40 2	30 9	1 92	0 4		
5L...	2 6Ro	0 0	2 0	18 8	16 1	1 75	-21 8		
6	2 6R2	0 0	0 3	33 2	24 0	2 19	-27 6		
6L ..	2 6R2	0 0	2 3	24 0	20 6	1 91	-18 0		
7 ...	2 6 r	0 0	0 0	14 9	- 1 1	1 87	24 1	23 7	2 30
7L....	2 6 r	0 0	2 0	9 0	6 0	1 15	-14 2	-12 6	
8	200 lb. KCl	0 0	0 0	11 0	0 6	3 48	14 9	9 3	4 67
8L ..	200 lb. KCl	0 0	2 0	27 0	10 6	2 40	- 9 7	-12 9	3 73
8b	200 lb. KCl	0 5	0 0	19.7	8 3	2 32	- 0 6		
8bL. .	200 lb. KCl	0 5	2 0	19 0	3 8	2 93	-45 2		

* Because amount of tissue was so small, No. 1 was preserved and analyzed with 1b, 1L with 1bL, 4 with 5, and 4L with 5L and the potassium content is given for these pairs together.

† r indicates raw shale; Ro indicates roasted at red heat for $\frac{1}{2}$ hour. R1 indicates roasted for $\frac{1}{2}$ hour at red heat with 0.5 tons CaSO₄. R2 indicates roasted at red heat for $\frac{1}{2}$ hour with 0.3 tons CaCO₃; L following a number indicates the part of the corresponding row treated with 2 tons of CaCO₃ per acre.

‡ A minus sign indicates less than control (applies to all the tables).

were preserved together because of the small amount of tissue in the oats, so no comparison can be made between roasting alone and roasting with gypsum as to content of potassium. However, the

percentage of potassium in the plants from the pots containing roasted shale is a little higher than in those from pots containing raw shale. Row 6 had the shale roasted with 11.5 per cent of its weight of CaCO_3 . Considering both limed and unlimed pots, the effect on growth of buckwheat is about the same as when the shale is roasted alone; but the potassium intake is about 10 per cent

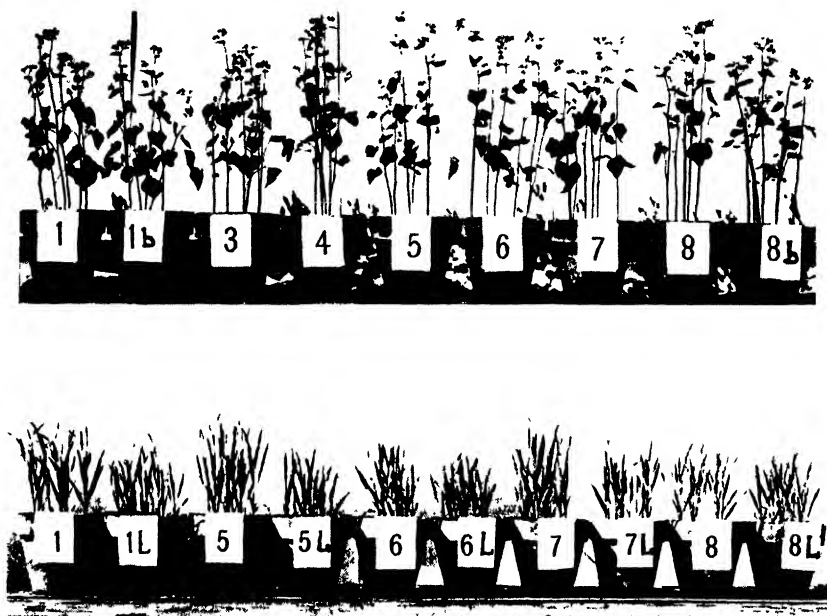


FIG. 8

higher. The oats were very sensitive to lime in this peat and were injured in the pots containing shale roasted with lime.

PLAINFIELD SAND

The results with alfalfa and winter wheat grown in Plainfield sand are shown in table V. Although not large, there is a rather consistent increase in dry weight due to shale in both of these sets of plants. Liming was beneficial in the crocks containing the wheat. Roasting the shale alone or with CaCO_3 or CaSO_4 seemed to have no effect on the growth of either set.

HAZEL CREST SAND

Table VI gives the results in Hazel Crest sand with alfalfa and clover. The alfalfa was benefited in only two rows out of six receiving shale, the best one gaining 17.2 per cent while the row grown on KCl increased 25.4 per cent. This clover grew in the same soil and pots as the alfalfa without further addition of fertilizers. It made rather large and consistent increases due to shale and KCl. This is

TABLE V

ALFALFA AND WINTER WHEAT GROWN IN PLAINFIELD SAND*; INCREASED GROWTH GIVEN AS PERCENTAGE OF GAIN OVER CONTROL

No	KCl (lb.)	SHALE (TONS PER ACRE)	CaSO ₄ (TONS PER ACRE)	ALFALFA†		WINTER WHEAT‡			
				Green (per- centage)	Dry (per- centage)	Green		Dry	
						No. Ca	2 T. Ca CO ₂	No. Ca	2 T. Ca CO ₂
1	0 0	0 0	0 0	Con- trol			11 7	.	0 00.3
2 . . .	0 0	0 0	0 52	11 1	10 1				
3 . . .	0 0	2 6 raw	0 0	13.4	11 3	9 6	10 6	24 4	20 6
4 . . .	0 0	2 6 roasted	0 0	10 6	7 7	1 6	35 2	1 9	18 0
5 . . .	0 0	2 6§	0 0	12 4	11 4	.		.	
6	0 0	2 6	0 52	12 6	12 3			.	
7	400 0	0 0	0 0	28 2	19 2	43 6	67 4	14 2	30 9..
8	400 0	0 0	0 52	28 2	17 7	

* Soil treated with 300 pounds of Ca(H₂PO₄)₂ per acre.

† All cans for alfalfa limed with 2 tons of Missouri limestone per acre, while one-half of crocks containing wheat treated similarly.

‡ All of the wheat crocks were given 200 lb of CaNO₃ 4 H₂O per acre

§ Roasted with 15 per cent CaCO₃

|| Roasted with 20 per cent CaSO₄

especially striking when one considers that it was obtained with a second set of plants on the same soil without any further addition of fertilizer after the first crop was removed. Using shale roasted alone and roasted with CaCO₃, both seemed to cause a little better growth than raw shale. Autoclaving and roasting the shale with CaSO₄ and autoclaving with CaCO₃ did not give any benefit over raw shale.

Very unexpected results were obtained in Hazel Crest sand with corn. When first tested for deficiency of potassium by growing buckwheat in it, a good response to potassium seemed to be obtained. But with corn (table VII and fig. 9) an enormously better growth was obtained (row 1) without potassium fertilizer and with only 2

tons of CaCO_3 per acre, as compared with even 400 pounds of KCl and 4 tons of CaCO_3 per acre. The potassium in this row 1 is only about 33 per cent of that in the control and 16 per cent of that in the best growth in KCl, while the weight of tops is nearly five times that of the control and more than twice that of the best growth in potassium chloride. This must indicate that there is sufficient available potassium in the Hazel Crest sand for corn, at least up to the

TABLE VI

ALFALFA AND CLOVER GROWN IN HAZEL CREST SAND, SOIL TREATED WITH 400 POUNDS OF $\text{Ca}(\text{H}_2\text{PO}_4)_2$, 4 TONS OF MISSOURI LIMESTONE, AND 416 POUNDS OF $\text{Ca}(\text{NO}_3)_2$ PER ACRE; INCREASED GROWTH GIVEN AS PERCENTAGE OF GAIN OVER CONTROL.

No	KCl (lb.)	POTASH SHALE (TONS PER ACRE)	CaSO_4 T PER ACRE	ALFALFA* (GREEN TOPS)	MEDIUM RED CLOVER†	
					Green tops	Dry tops
1 . . .	0	0 0	0 00	Control
2 . . .	0	0 0	0 52	— 0 5	12 2	7 2
3 . . .	0	2 6 raw	0 00	— 5 3	25 3	29 1
4 . . .	0	2 6 roasted red heat $\frac{1}{2}$ hr.	0 00	— 3 8	32 5	37 8
5 . . .	0	2 6 roasted with 0.52 T. CaCO_3	0 00	— 0 5	40 5	38 9
6 . . .	0	2 6 autoclaved‡ with 0.52 T. CaCO_3	0 00	12 9	29 7	23 4
7 . . .	0	2 6 roasted with CaSO_4	0 52	1 9	17 2	15
8 . . .	0	2 6 autoclaved‡ with 0.52 T. CaSO_4	—	17 2	16 9	14 9
9 . . .	400	0 0	0 00	25 4	83 8	66 0
10 . . .	400	0 0	0 52	16 3	65 2	48 3

* Alfalfa grew 17 weeks and clover 19 weeks, from January to May

† Grown in same soil and cans as alfalfa of previous year, without addition of fertilizer

‡ Autoclaved at 15 lb. steam pressure 30 minutes.

age attained in this experiment. Precipitated CaCO_3 must be injurious for corn in this soil when used at the rate of 4 tons per acre. The benefits seemingly due to potassium fertilizers in this experiment must have been due to their corrective effects on lime injury. The carbohydrate-nitrogen ratio, where there was no lime injury, is seen to be about twice that of the best row injured by lime.

Discussion

BENEFITS OF RAW SHALE

From the foregoing tables it is seen that 2.6 to 3 tons of untreated shale per acre benefited most of the plants grown in potash-deficient

TABLE VII

CORN GROWN IN HAZEL CREST SAND; INCREASED GROWTH GIVEN IN PERCENTAGES OF CONTROL; K IN PERCENTAGE OF TISSUE. SOIL TREATED WITH 300 POUNDS OF $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot 2\text{H}_2\text{O}$ AND 416 POUNDS OF $\text{Ca}(\text{NO}_3)_2$ PER ACRE. ALL ROWS LIMED AT RATE OF 4 TONS OF CHEMICALLY PURE PRECIPITATED CaCO_3 PER ACRE EXCEPT NO. 1, WHICH WAS GIVEN ONLY 2 TONS

No.	POTASH SHALE (TONS PER ACRE)	CaSO ₄ (TONS PER ACRE)	GROWTH OF SHOOTS		K PER CENT	NITROGEN PER CENT				CARBOHYDRATES				
			Green	Dry		Insol. Org.	Soluble Org.	NH ₃ + NO ₃	Tot N	Reduc- ing	Non- reducing	Poly- sac	Total	Carbo- hydrate N
1. . .	0 0	0 0	653 7	385 7	0 63	1 83	0 32	0 49	2 64	9 37	1 42	21 9	32 7	12 4
2. . .	0 0	0 0	6 6	lost										
3. . .	0 0	1 1	Control		1 75	1 73	0 72	1 81	4 26	2 04	0 35	20 9	23 3	5 47
4. . .	3 0 raw	0 0	1 5	0 0	1 72	1 87	0 00	2 83	4 70	2 34	2 09	16 5	20 9	4 45
5. . .	3 0 Ro*	0 0	— 5 9	6 5	1 71									
6. . .	3 0 Ro(1)	0 0	— 26 5											
7. . .	3 0 Auto. (1)	0 0	19 0	lost										
8. . .	3 0 Ro(2)	1 1	66 2	51 9	1 01	1 89	1 22	1 62	4 73	2 53	3 20	16 5	22 3	4 92
9. . .	3 0 Auto (2)	1 1	65 4	51 5	1 58	1 68	1 15	1 25	4 08	2 12	1 04	19 4	22 7	5 56
10. . .	221.0 lb. KCl	0 0	128 7	100 0	3 46	1 65	0 93	0 90	3 48	2 17	3 17	16 5	21 7	6 24
11. . .	221.0 lb. KCl	1 1	219 9	138 8	3 36	1 83	0 95	0 78	3 56	4 57	1 01	18 3	23 9	6 72
12. . .	256.0 lb. K ₂ SO ₄	0 0	114 7	80 8	3 70	2 05	0 98	1 10	3 13	1 44	0 14	19 3	20 9	6.67
13. . .	300 lb. KNO ₃	1 1	39 3	— 0 3	4 95	2 64	1 51	0 63	4 78	3 16	2 24	15 6	21 0	4 39

* Ro indicates roasted at red heat for 30 minutes; Ro(1) indicates roasted with 20 per cent CaCO_3 ; Auto (1) indicates autoclaved at 15 lb. steam pressure for 30 minutes with 20 per cent CaCO_3 ; Ro(2) indicates roasted with 11 ton of CaSO_4 ; Auto (2) indicates autoclaved with 11 tons of CaSO_4 .

soils. These increases varied from 7 to 25 per cent. The seeming exceptions were in the Hazel Crest sand which apparently had sufficient available potassium for corn. Analysis showed that this sand contained about seven times as much potassium as the University of Wisconsin peat and two and a half times as much as the Plainfield sand. Clover made a gain of 253 per cent, but some of this increased growth may have been due to a slight amount of sulphur in the shale. Of four sets of plants analyzed for potassium and grown on potash-deficient soils, three showed the following increases in percentages of potassium due to raw shale: corn 5 per cent, oats 63 per cent, and buckwheat 100 per cent. In general the gains were larger on the



FIG. 9

peats than on the sandy soils. This may have been due to the peats being more deficient in available potash, for they contained a lower percentage of potassium than the sandy soils used (table I).

ROASTED SHALE

Tomatoes and corn made the large increases of 61.7 and 62.1 per cent respectively on quartz sand with roasted shale. However, raw shale was not tried in the quartz sand for comparison. Corn in the University of Wisconsin peat, buckwheat in the Coddington peat, and red clover in the Hazel Crest sand showed from 25 to 250 per cent more gain with roasted than with raw shale, while corn and clover contained approximately 50 per cent higher of potassium when grown on the roasted shale. Analysis showed that raw shale contained 0.065 per cent of water-soluble potassium while roasted shale contained 0.094 per cent, or about 45 per cent more than raw shale; hence it seems that roasting increases by nearly one-half the availability of the potassium in the shale.

GYPSUM AND SHALE

The effect of using gypsum with potash shale in quartz sand for tomatoes is shown in table VIII. While all four rows show gains, only two of them are great enough to be of significance. These plants were grown in a hot greenhouse in summer, and some of the benefit may have been due to the increased water-holding capacity of the sand caused by the finely powdered gypsum. In the University of Wisconsin peat the gypsum was merely mixed with the shale and not roasted with it. No benefit was shown in the corn, but the clover

TABLE VIII

TOMATO PLANTS GROWN IN QUARTZ SAND WITH SHALE PLUS GYPSUM; INCREASED GROWTH GIVEN AS PERCENTAGES OF GAIN OVER CORRESPONDING ROWS GROWN WITHOUT GYPSUM

No	SOLUTION USED	SHALE (TONS PER ACRE)	CASO ₄ (TONS PER ACRE)	GROWTH GAIN, PERCENTAGE OVER NO CASO ₄
2 ..	Shive's without K	0 5	0 5	4 7
3 ...	Shive's without K	1 5	1 5	20 6
4 ...	Shive's without K	3 0	3 0	2 7
5 ...	Shive's without K	6 0	6 0	23 6

made a much larger growth due to the presence of sulphur. However, the clover also showed a 25 per cent increase in content of potassium. The tomato plants grew considerably better when shale was mixed with gypsum, but no analyses were made for potassium. With the Coddington peat, when gypsum was roasted with shale no effects were manifested in growth, but one 35, and one 50 per cent gain in potassium was found in buckwheat. The effects of the gypsum in the Plainfield sand were too small to be of significance, and the increases in growth in Hazel Crest sand, as already indicated, cannot be taken as due to available potassium. The corn plants grown in the row treated with shale roasted with gypsum, however, showed about 10 per cent higher potassium than the row treated with shale roasted alone. Analysis of the shale indicated 0.094 per cent of water-soluble potassium in the roasted shale and 0.124 per cent in that roasted with gypsum. Thus roasting with gypsum increased the content of soluble potassium about twice as much as

roasting without it. Autoclaving the shale and gypsum at 20 pounds steam pressure for 30 minutes seemed to have no effect on the availability of the potassium in the shale. From the growth results in the first three soils, from the increase in the percentage of potassium in the plants, and from the analysis of the treated shale, it seems that roasting with gypsum nearly doubles the amount of available potassium in the potash shale.

SHALE AND CALCIUM CARBONATE

No shale that had been roasted with CaCO_3 was used in the quartz sand or in the University of Wisconsin peat although this was tried in the other soils. Oats were so sensitive to the lime in the Coddington peat that the small amount used with the shale caused injury. The buckwheat in this peat did not seem to be affected in growth but contained 9 and 15 per cent higher potassium content when the shale was roasted with CaCO_3 than when it was roasted without it. The red clover grown on Hazel Crest sand showed about 25 per cent gain in dry weight due to roasting the shale with CaCO_3 . Analysis showed that the untreated shale contained 0.064 per cent of water-soluble potassium, the roasted shale 0.094 per cent, shale roasted with 20 per cent of its weight in CaCO_3 0.105 per cent, and shale roasted with 33 per cent of its weight in CaSO_4 0.124 per cent. The results indicate that roasting with CaCO_3 increased the availability of the potassium in the shale a little more than the roasting alone, but not as much as roasting it with gypsum. None of the results obtained, however, showed that autoclaving at 20 pounds pressure for 30 minutes has any effect on the availability of the potassium in the shale.

SAP ACIDITY AND UTILIZATION OF POTASSIUM

TRUOG (32) believes that plants with neutral or nearly neutral sap have greater ability to feed on difficultly soluble forms of potassium than plants with more acid sap. It will be interesting to see if these results bear out that theory. There are three bases on which such a comparison may be made: (1) the percentage of gain over the control; (2) the percentage of potassium in the plants; and (3) the total amount of potassium taken in. To obtain plants with differ-

ent hydrogen-ion concentrations growing upon the same soil, would require the use of different kinds of plants. Different species of plants will have different potassium requirements, different rates of growth, and different degrees of response to potash fertilizers. It seems that the best means of comparison would be by ratios, obtained for each kind of plant by comparing the results on shale with the results with KCl. The ratio used in comparing growth was found by dividing the percentage of gain over the control obtained on shale

TABLE IX

COMPARISON OF ABILITY OF PLANTS TO OBTAIN POTASSIUM FROM RAW SHALE

PLANT	SOIL	PER- CENT- AGE GAIN, 2 6 T SHALE	PER- CENT- AGE GAIN ON KCl	RATIO OF GAINS	RATIO OF PERCENT- AGE OF K	RATIO TOTAL K	PERCENT- AGE K*	SAP PH
Corn	U. of Wis. peat	16 4	68 0	0 25	0 35	0 25	2 07	5 19-5 52
Clover	U. of Wis. peat	253 8	380 9	0 67	0 30	0 22	1 36	5 6 -6 19
Buckwheat	Coddington peat	14 9	19 4	0 77	0 53	0 53	1 87	4 18-5 00
Oats	Coddington peat	24 1	14 9	1 62	0 49	0 56	2 03	5 58-5 70
Winter wheat	Plainfield sand	16 6	67 4	0 25	No anal- yses	No anal- yses	No anal- yses	6 12-6 33
Alfalfa	Plainfield sand	13 4	28 2	0 48	No anal- yses	No anal- yses	No anal- yses	6 19-8 25

* In plants grown on shale.

by that secured with KCl for the same kind of plant grown under otherwise comparable conditions. The other two ratios were computed similarly for each kind of plant used. Table IX presents these ratios for three pairs of rows of plants. The two kinds of plants in each pair were grown on the same soil but have different sap acidities. Each pair was grown on a different soil. The sap acidities given were taken from different investigators and give the maximum range recorded for each variety of plant. The gains in tissue weight and in the percentages of potassium for the shale are based upon plants from the rows of untreated shale, because these were found to contain the least quantity of available potassium, and we are at-

tempting to compare the ability of the plants to take up potassium from a difficultly soluble source. The ratios for the percentages of potassium, and for the totals of potassium, are so nearly alike in the pairs that they do not seem to indicate any relation between the amount of potassium taken up and the acidity of the sap. However, the ratios between the gains on shale and the gains on the soluble potassium salt are approximately double in every case with plants having the more nearly neutral sap. This seems to agree with TRUOG's theory only in part, for the nearly neutral sap plants take up no more potassium from the shale than those with the more acid sap, but make better use of what they get, that is, they thrive better on the limited supply.

LIME INJURY

It has long been known that certain peat and muck soils are injured by excessive liming. LOEHWING (16) thinks that it is due in part to the depression of the intake of potassium in the presence of the lime, and in part to the precipitation of iron in the tissues (17). Two of the soils used in these experiments were injured by excess of CaCO_3 , the Coddington peat and the Hazel Crest sand. Sandy soils which are injured by lime are very rare, but this one with a loss on ignition of only 7.1 per cent exhibits the phenomenon usually attributed to mucks and peats. In the Coddington peat (table IV) the oats average 23 per cent higher and the buckwheat 33 per cent higher in potassium where no lime was used than in the limed pots; so apparently lime restricts the amount of potassium taken up by the plants. On the other hand, some of the pots showing reduced growth in the presence of lime have a larger percentage of potassium than others making a much better growth without lime. For example, in the corn grown in Hazel Crest sand (table VII), the row given 2 tons of CaCO_3 to the acre produced nearly five times the weight of dry tops that was grown by the row receiving 4 tons. The uninjured row contained only 0.63 per cent of potassium while the injured row contained 1.74 per cent, or nearly three times as much. Evidently it was some other cause than potassium deficiency that produced the lime injury in both of these soils.

The percentage of ammonia plus nitrate nitrogen in the rows of corn injured by the lime is from two to four times as great as it is in

the uninjured row, while the carbohydrate-nitrogen ratio is only about one-half as large in most of the injured rows as it is in the unharmed row. This agrees with LOEWING's results in mucks and peats already referred to, and it may indicate, as he suggests, a reduction of carbohydrate synthesis, which in turn checks nitrate assimilation. BOBKOW and others (5) found some uncultivated soils, especially light ones, injured by excessive liming. They report that a pH 8 was produced, together with large quantities of ammonia, nitrites, and nitrates. They say that the injury was removed when the ammonia was nitrified.

RESIDUAL EFFECTS OF SHALE

Three series of plants were grown in pots of soil from which a crop had been removed and to which no other fertilizer had been added.

TABLE X

TOMATO PLANTS GROWN IN SAME PEAT AS FOR CORN IN TABLE III,
WITHOUT FURTHER ADDITION OF FERTILIZER

No.	FERTILIZER PER ACRE			GAIN OVER CONTROL (PERCENTAGE)	
	Shale (tons)	KCl (pounds)	CaSO ₄ (tons)	Green	Dry
1a . . .	0 0	0 0	0 0	Control	
1b	0 0	0 0	1 3	— 8 0	— 14 1
2	0 43	0 0	0 0	— 10 8	— 30 6
3a	1 3 R*	0 0	0 0	3 0	— 11 7
3b	1 3 R	0 0	1 3	23 5	5 9
4a	2 6 R	0 0	0 0	18 2	8 2
4b	2 6 raw	0 0	0 0	13 4	14 9
5	5 2 R	0 0	0 0	82 9	81 5
6a	0 0	218 0	0 0	217 7	164 7
6b	0 0	218 0	1 3	164 1	105 7

* R indicates roasting at red heat for 30 minutes

The first crops had not grown long enough thoroughly to test the residual effects of the fertilizers, but some indications of these effects are seen. The tomato plants of table X followed corn which had grown 11 weeks. The tomatoes made no gains over the control in the lowest two applications of the shale. Two other rows showed rather low increases in weights, suggesting that perhaps most of the available potassium in this soil had been taken up by the corn. However, the highest applications of shale (5.2 tons) and the 218 pounds of KCl

per acre produced about 50 per cent greater gains on the shale and more than twice the increases with KCl than was obtained with the tomatoes receiving original fertilizer treatments in the quartz sand. This must indicate that sufficient potassium remained in the rows of highest shale application and in those receiving the KCl, but not in the other rows. Soy beans were grown on this same soil after the tomato plants had grown 12 weeks and had been removed. They showed an increase of 17.1 per cent on the KCl row. With the 5.2 tons of shale no benefit was shown, probably because of wilt injury that was not visible on the other rows. Five out of six of the remaining rows made gains of 5-10.6 per cent.

The red clover (table VI) was grown on Hazel Crest soil after alfalfa without further fertilizers. It showed much better responses to the potassium fertilizers than did the alfalfa that preceded it. This better growth may have been due to two things. In the first place, the alfalfa may have been injured by excess liming and this effect may have disappeared during the interval between the crops. The soil stood in a dry place about 8 months before it was used the second time. The second reason may be that red clover responds more vigorously to potash fertilizer than does alfalfa. While the residual effect on the clover seems high, this response in the lower applications of shale with tomatoes, and with all of the shale-treated rows of soy beans, is somewhat meager. In all cases the residual effect of the KCl far exceeded that of the shale, in spite of the fact that 2.6 tons of shale to the acre added approximately twice as much potassium to the soil as did the KCl used. This seems to indicate that after the more available potassium in the shale has been used by the plants, the remainder becomes available very slowly under the conditions of the experiment. Possibly under field conditions it might become available more rapidly. After this shale had been heated to white fuming temperature with concentrated sulphuric acid for 30 minutes, only 12.4 per cent of the potassium in it was water-soluble, while 62 per cent in the shales from Union County, Illinois, and 60 per cent of the potassium in the Decorah shale of Minnesota were made soluble by this treatment. The Cartersville shale evidently contains a much smaller amount of readily available potassium than do these other two shales.

EXCESSIVE ABSORPTION OF POTASSIUM

GODELEWSKI (12), studying the potassium content of plants with regard to a sufficient supply in the soil, thinks that 2 per cent in the tissues of cereals and legumes indicates an abundance in the soil and hence in the tissues. In table III, corn is seen to have as high as 6.3 per cent of potassium, while it made nearly as good growth with 3 per cent. Clover showed its best increase with 2.6 per cent of potassium in its tops, while grown with KCl it contained 4.6 per cent. Buckwheat (table IV) showed its best weights with 1.92 per cent potassium, while its maximum percentage when fertilized with KCl was 4.67. The minimum figures here are most of them higher than GODELEWSKI's, but his analyses were made on older plants and the percentage may decline as the plants mature. These maximum figures for potassium indicate that the plants grown on soluble KCl took up 40-100 per cent more than they needed at the stage of development at which they were cut. It seems probable that a less soluble form of fertilizer, in which potassium became available only as needed by the plant, would be less wasteful of this element.

GEORGIA SHALE AS FERTILIZER

These potash shales of Georgia are very favorably located, near the sands and peats of the coastal plain of the southeastern states, many of the soils of which are deficient in potash. Fifty per cent of our imported potash is said to be used in the four states North Carolina, South Carolina, Georgia, and Alabama (30). TURRENTINE (33) states:

These shales are soft and do not increase in hardness to any extent with depth. . . . They can be mined with ease and at low cost. . . . The chemical character of the material does not vary after a depth of from 5 to 10 feet has been reached. . . . The climate is favorable for open cut mining throughout the year. . . . The strata carry an average of 8 to 9 per cent potash. . . . Being hydrous silicates, they are decomposed much more readily than anhydrous silicates e.g. orthoclase feldspar.

From these considerations of convenient location and ease of mining and processing, from the response to potash of the plants grown with the shale in these experiments, and the increases in potassium content of the plants due to shale, it seems that Georgia shales should form a valuable fertilizer in applications of 2 tons or more

per acre. Heating with gypsum could be employed with soils needing sulphur, as some peats evidently do, and calcining with CaCO_3 could be practiced for soils needing lime. Crops making large responses to the potassium in the shale, such as red clover, could be used in rotation; and the sod, when turned under, would contain considerable potash rather readily available to plants which do not secure sufficient potash from the shale itself. It was hoped that alfalfa would prove a satisfactory substitute in regions where red clover does not thrive, but the results in these experiments do not indicate it. Crimson clover was tried with the Hazel Crest sand, but this soil contained so much available potassium that no decisive results were obtained from the use of the shale.

Summary and conclusions

1. Twelve kinds of plants were grown in pots in quartz sand and in four different soils with potash shale, potassium chloride, and Shive's solution as sources of potash.

2. Green and dry weights were obtained, and considered, when compared with the controls, to indicate the benefit derived by the plants from the potash shale and from the more soluble forms of potassium.

3. The tops of a part of the plants were analyzed for potassium, showing in certain cases 60–100 per cent increase in potassium content when grown with applications of 2.6 tons per acre of untreated shale; much larger increases occurred with KCl applications.

4. The use of untreated shale showed that considerable benefit is derived from it by plants grown in potash-deficient soils, especially peats.

5. Shale roasted at red heat for 30 minutes produced greater responses than untreated shale, and was found to contain 45 per cent more water-soluble potassium. When roasted with 20 per cent of CaCO_3 it produced a little better response than when roasted alone, and showed 12 per cent more water-soluble potassium. Autoclaving the shale with 20 per cent CaCO_3 at 20 pounds steam pressure for 30 minutes did not increase the amount of water-soluble potassium over that in the untreated shale. Roasting with 37 per cent gypsum for 30 minutes at red heat gave still better growth of

plants, and increased the water-soluble potassium 30 per cent more than roasting it alone. Autoclaving the shale with gypsum at 20 pounds steam pressure for 30 minutes was of no benefit.

6. Pairs of rows of different kinds of plants grown in the same soil were compared on the basis of the ratio of the gain on raw shale to the gain on KCl. The plants with more nearly neutral sap received about double the benefit from the shale that was received by plants with more acid sap.

7. One peaty soil and one sandy soil were injured by excessive applications of CaCO_3 , but the harm did not seem to be due to the depression of the intake of potash in its presence. Corn grown in this sandy soil was analyzed for carbohydrates and nitrogen. Results showed that the carbohydrate-nitrogen ratio was nearly twice as large when there was no lime injury, as in the case of lime-injured plants. The low percentage of carbohydrates and the high percentage of ammonia plus nitrate nitrogen suggest a checking of photosynthesis and consequently of the utilization of inorganic nitrogen. The depression of photosynthesis may be related to iron immobility in the over-limed plants.

8. Residual effects obtained by growing two or three crops in the same pots of soil without additional fertilizer indicated that, after the most readily available potassium was taken up, the remainder became available very slowly under greenhouse conditions.

9. The shale was heated with concentrated H_2SO_4 to fuming temperature for 30 minutes. Then only 12.4 per cent of the potassium in the shale was water-soluble as compared with 62 per cent of the Union County shales of Illinois and 60 per cent in the Decorah shale of Minnesota. The low solubility with H_2SO_4 and the responses with plants indicate that the potassium in this shale is considerably less available than in the other two shales.

10. The plants grown on soil treated with KCl contained 1.5-2 times as much potassium as was found in other plants of the same kind, growing almost if not equally as well, and fertilized with potash shale.

11. Shale, roasted with limestone or gypsum, would probably prove a valuable fertilizer in applications of 2 tons or more per acre, especially on peaty soils and for certain crops like red clover. Plot experiments in the field, however, are needed to test it further.

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EFFECT OF MOISTURE SUPPLY ON DEVELOPMENT OF PYRUS COMMUNIS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 406

ALDEN F. BARSS

(WITH ELEVEN FIGURES)

Introduction

A number of interesting investigations have been carried on in the past in an effort to discover the effects of different environmental factors upon plant development. In recent years many of these studies have dealt with plants of agricultural or horticultural importance.

Much information has been gathered through these investigations as to the effect on plant development of such factors as light, temperature, air humidity, soil acidity, and various chemical substances. Other studies attempting to discover the relation of soil moisture to plant growth have included, among other things, estimations of the total water requirements, determinations of the wilting coefficient of various soil types, and the effect of atmospheric conditions on the ratio between the number of pounds of water used and the number of pounds of dry matter produced. So too, many irrigation experiments have been carried on with fruit trees in the orchard districts of the west, limited mainly to studies of the effect of applying water by various systems and in varying amounts on the fruit itself or on the total crop produced. Most if not all of these investigations have not recorded the actual effect of the changes in water supply upon the tree itself or upon its fruiting habits.

In order to understand this fundamental phase in the larger problem of commercial irrigation, as well as to supplement general knowledge of plant behavior, an investigation was commenced in December, 1912, for the purpose of determining the effect of varying amounts of moisture upon the growth response and tissue development of the pear tree. A preliminary report (2), showing certain

gross effects of supplying different amounts of water under controlled conditions, is here supplemented with additional records, including the results of a microscopical study of the changes in the anatomy of pear shoots as influenced by water supply.

Material and methods

To avoid the inexactness of outdoor conditions the investigation was carried on in the greenhouse. Forty-eight uniform Bartlett pear trees, dwarfed on Angers quince roots, three years old from the bud, were transplanted into rich soil (half composted horse manure and half garden loam) into 12-inch pots, a layer of $\frac{1}{2}$ inch of quartz sand placed on top of the soil, and the pots set into shallow pans 'to prevent loss of water by percolation. These forty-eight trees were then divided into four lots of twelve each. These lots received identical treatment in every respect save one, the application of water.

All the water was measured. By applying enough water to the trees in lot L so that there was seepage in the pans in which the pots were standing, these trees were assured a continuous supply. The trees in lot F were given the same total measured amount of water as those in lot L; but were given only one-half the amount at each application and watered approximately twice as frequently. The trees in lot M received half as much water as those in lot L (or F) by watering them every second time that the trees in lot F received their quota. The remaining twelve trees, making up lot S, received the smallest amount of water possible to retain them alive.

Only during the first season was this procedure departed from, and then only to the extent that during the last three months of the growing season the trees in lot F were given much less water than those in lot L. It was also found that occasionally the watering of certain individual trees, especially in lots L and F, had to be varied somewhat in order to maintain the same general relation between the trees in the different lots. Trees which were already supplied with all the water they could use could not be forced to take in more by applying an excess.

The total water application for each tree during the first growing season of 7 months was as follows: lot L (large amount) $49\frac{1}{2}$ quarts

to each tree; lot F¹ (frequent and large) 37 quarts to each tree; lot M (medium amount) $17\frac{3}{4}$ quarts to each tree; lot S (small amount) 11 quarts to each tree.

Since soil moisture determinations were not made during the progress of the experiment, there is the probability that the actual moisture content of the soil varied among the different trees in any one lot. Between the separate lots, however, the relative amount of water would not vary a great deal, and it is thought would rather closely approximate moisture relations that could be maintained under field conditions.

The total water application for each tree during the second growing season of 8 months was: lot L, $72\frac{1}{2}$ quarts; lot F, 69 quarts; lot M, 38 quarts; lot S, 15 quarts. And during the third season of 7 months: 57 quarts, 58 quarts, 30 quarts, and $7\frac{1}{2}$ quarts respectively in the four lots L, F, M, and S. Each year it was possible to maintain approximately the same ratio of watering between the different lots. At the beginning of each season all trees in all lots were given a thorough watering to start them into growth.

Each spring all open blossoms were artificially pollinated by means of a camel's hair brush with pollen from a Winter Nelis tree which had been brought in to use for this purpose.

Throughout each growing season records were made from time to time of the general appearance, habit and amount of growth, bud formation, flowering and fruiting of the trees in the four lots.

Samples of the wood growth were taken from the trees at various times to study the effect of the different watering on the tissue development. Such samples for histological purposes were taken the first year: (1) at the beginning of the experiment; (2) when growth was well started; (3) one month later; (4) three months after the previous collection; and (5) while the trees were dormant just before growth started for the second season (this would be similar to no. 1 but a full year later). These wood samples of the current growth, as representative as possible of the trees from which they were taken, were cut transversely into small blocks about an eighth of an inch long, killed and fixed in formalin-alcohol; desilicified when necessary

¹ During the last 3 months of the season, lot L received 63 per cent more water than lot F.

with hydrofluoric acid; infiltrated with celloidin; sectioned; stained with safranin and Delafield's haematoxylin, and mounted in Canada balsam on glass slides, the system followed throughout being largely that outlined by CHAMBERLAIN (5).

No chemical analyses or microchemical tests were made.

General growth studies

As is natural with any growing trees, there were rather wide fluctuations within each lot of twelve, as also between parts of a single tree. It was not to be expected that a given treatment would result in all individuals so treated producing the same number of branches of uniform length and diameter, nor an identical number of flower clusters setting and maturing the same number of fruits of uniform size. The removal of samples at various times had the effect of pruning on the remainder of that branch, with varying results depending upon the growth stage of the trees at the time the samples were taken. The bearing of fruit by certain trees in any one year and not by others also resulted in whole trees or parts of trees in a single lot giving changed responses the year following from those trees which bore no fruit. For these reasons it seems unnecessary to present full tabulations of the figures secured, but attention is drawn to the definite facts and outstanding differences which became evident during the course of the investigation.

1. FREQUENCY OF WATERING.—In no respect did the results obtained in either of the two heavily watered lots show a material difference over those of the other. Under the conditions of this particular experiment, therefore, lot L with a large amount of water and lot F with approximately the same total amount, but applied twice as frequently in half the quantity at a time, may be considered practically identical.

2. STARTING GROWTH.—The previous year's difference in watering appeared to have no influence on the time of starting growth the next season (fig. 1, upper).

3. FLOWER CLUSTERS.—Except for the first spring when there was no bloom, practically every tree in every lot produced some blossoms every year. The trees receiving the large supply of water produced during the first two years of blossoming the greatest number of

cluster buds (buds containing a cluster of flowers, leaves, and vegetative growing points within the one set of bud scales). In the second of these years, these trees exceeded those in the moderately watered



FIG. 1.—Comparison of representative trees, one from each lot, during second year of experiment; trees arranged from left to right according to water supplied: L, large amount; F, frequent and large amount; M, moderate amount; S, small amount: upper, during active growth; lower, nearing end of growing season.

lot by having almost double the number of blossoms to each tree. In the third year the situation was reversed, however, lot M trees producing almost twice as many cluster buds to each tree as those in lot L or lot F, which lots the season previous matured the greatest number of fruits. The scantily watered lot at no time equaled the others in number of cluster buds.

4. FLOWERS.—The trees in lot S in its first year of bearing averaged the greatest number of flowers to each cluster, due to the fact that a high percentage of its clusters were borne on spurs (79 per cent), whereas the heavily watered lots had almost half their clusters (45 per cent) borne laterally on the previous season's growth; such axillary clusters average fewer flowers to each bud than do spur or terminal clusters. Lot M averaged between the other two in this respect.

5. SETTING OF FRUIT.—No effect on fruit setting seemed attributable to the watering except in lot S, with least water, where the first year showed 100 per cent fall of flowers, no fruit maturing, and a very low percentage maturing the next year. In the other lots the fruit setting seemed to be largely a matter of individual tree performance. A heavy set of fruit one year was followed by a light set or none the next year.

Abscission of such fruits as did not mature was noticeably different in the lot given the least water as compared with that in the others. In the former the fruits, a dull, grayish-green color, remained small and were limp during a large part of the day, hanging shriveled for several days before finally separating from the tree. In the other lots the fruits which fell were of good size and to all appearance fertilization and early development had been normal, with a full quota of plump seeds forming in most specimens examined. The dropping of these fruits came as a distinct cutting off or separation preceded by a pronounced yellowing at the point of attachment between stem and cluster base, the fruits falling of their own weight, while still a clear green and turgid.

6. MATURED FRUITS.—The number of fruits matured, especially where more than one was borne on a single tree, seemed to have had a greater effect on the size of the individual fruits and on the crop the following year than did the actual amount of water (table I).

It is evident that with a small crop even the trees receiving a medium amount of water were able to produce full-sized fruits, averaging much larger than those in the heavily watered lots, which were maturing more fruits that year. When the size of crops borne in these lots was reversed, as in the first year, the average size of fruit was reversed (figs. 2-7). It is worthy of note in this connection, however, that in the year of this large crop, the trees given a moderate amount of water produced much smaller fruits on the average than did the heavily watered trees in the year of their large crop. Watering, then, pronouncedly influenced the size of the fruits under the conditions of this experiment.

TABLE I
FRUIT RECORD

Lot	PERCENTAGE CLUSTERS MATURING FRUIT		NO OF TREES MATURING FRUITS		NO OF FRUITS MATURED		AVERAGE WEIGHT OF EACH FRUIT (GM)		AVERAGE SPECIFIC GRAVITY	
	Year		Year		Year		Year		Year	
	1	2	1	2	1	2	1	2	1	2
L	16 0	18 0	6	10	12	50	202 6	138 8	0 9042	0 984
F	9 3	18 3	4	10	7	49	236 9	147 4	0 9958	0 987
M	33 3	6 4	7	7	20	9	93 5	174 4	1 0411	0 9915
S		4 5		2		3		41 6		1 08

For the first crop, it may be said that at the time of harvesting and when ripe, the fruits in the lot given a medium amount of water differed from those in the heavily watered lots by being not only smaller, but showing less the characteristic uneven ribbing of the surface. The color was at first a dull bluish-green instead of a clear lively green, which later, however, changed to the normal yellow on ripening. They also lacked the crisp firm feel to the touch. When ripe these fruits in lot M had a tough skin and flesh, and such poor flavor and quality as to make the fruit decidedly undesirable for eating. The following year, however, lot M, with a small crop as compared with lots L and F, bore fruit of the usual high quality normal for the Bartlett variety, just as did lots L and F with many more fruits maturing. The fruits in lot S were small, poor flavored,

gritty and astringent even when ripe. The watering noticeably affected not only the size of the fruit, but their flavor and quality, especially in a large crop year when the water supply was limited.



FIGS. 2-4.—Crop produced first fruiting year under varying amounts of water: upper (lot L), large amount; center (lot F), frequent and large amount; lower (lot M), moderate amount.



FIGS. 5-7.—Crop produced second fruiting year, under varying amounts of water: upper (lot L), large amount; center (lot F), frequent and large amount; lower left (lot M), moderate amount; lower right (lot S), small amount.

7. VEGETATIVE GROWTH.—Probably the most noticeable variation in response to the application of different amounts of water was in the nature and amount of vegetative development. Considering as a shoot any new growth of the current year 4 cm. or over, it was found each year that the heavier the watering the greater were these shoots in number, length, average diameter, number of nodes, number of leaves to each shoot, and average size of leaves. The length of the growing season was also much greater. The scantily watered trees were lowest in all these features, the moderately watered trees ranging midway between the extremes (fig. 1, lower).

In the heavily watered lots the first growth in each season was frequently followed by a second or even a third growing with little cessation between the growths. The other lots had a much shorter period of first growth, however, and where any second growth came it followed a period of several weeks during which the trees seemed to have gone into a state of partial dormancy. In the fruiting years a renewed period of vegetative activity followed harvesting of the fruit, such new growth being limited largely to the trees, branches, and cluster bases from which the fruits had been picked. Again such response came occasionally from the bud just below the place from which a branch had been removed for histological study.

An indication of relative tree size is shown in the average circumference of the trunk taken at the end of three seasons of growth under the varying treatments accorded: lot L, 70.9 mm.; lot F, 68.3 mm.; lot M, 60.2 mm.; lot S, 55.5 mm.

8. RELATION OF SHOOT GROWTH TO FRUIT. —There appears to be a close qualitative and quantitative relationship between shoot growth and fruit development which seems to account for the great variations which occurred within each lot, even when all the trees in the lot had been given the same treatment. The general tendency for flower and fruit development to be greatest where shoot growth was least and vice versa was pronounced in all four lots, a correlation which has been noted and discussed by several workers in connection with their own investigations. Again it was found that the largest fruits in any one lot were generally on a tree where fewest fruits were matured, the smallest average weight to each fruit being usually where most fruits were matured.

The results secured with these trees would seem to indicate that under the conditions of this experiment, even though watering appeared to be able to alter in a measure the degree of expression as shown by number and length of shoots, number and size of fruits, nevertheless growth and fruiting appeared to have a direct interrelation over and above the effects produced by the different amounts of water applied. That is to say, by heavy watering it was not found possible to force all trees so treated into a condition of extreme vegetation with no flowering, or by medium watering into a condition of moderate vegetation and heavy fruiting, or by scanty watering produce trees which neither grew branches nor formed flowers.

A further observation may here be recorded, although its significance is uncertain, since it is realized that tree records from different seasons do not lend themselves to exact comparison.

The third growing season, although the trees in lots L and F were older and larger than they were the year before, they actually used on the average considerably less water to each tree than they did the previous year, even though at all times given as much water as they would take up. Heavy fruiting and a lower total shoot growth were associated with this lessened water consumption. These conditions might suggest that a large fruit crop makes less demand on the water supply than does a large shoot growth with its extensive leaf surface.

Histological studies

In order to make possible a direct comparison of tissue development in wood sections from the different trees at the several dates, a typical section was selected on each slide which represented a distinct branch area (near the top of the new growth, at the center, or near the base), and camera lucida drawings with uniform magnification were made of these sections. By means of some four hundred such drawings, three representative examples of which are shown in fig. 8, a careful study was made of the gross topography of the main tissues, to see the proportionate amount and placement of these tissues in the trees of the four lots L, F, M, and S.

This grosser microscopical study was followed by a more detailed microscopical examination of the separate cells as seen in cross-section; noting the proportion of the different kinds of cells existing

in the several tissues, the size of the individual cells, and the thickness of their walls in comparative regions on branches from the different lots. Figs. 9-11 show representative camera lucida drawings used in this study.

That the trees were satisfactorily uniform at the starting of the experiment was evident from an examination (from the sectioned material) of the growth of the previous year, taken at the time of transferring the trees to the greenhouse, December 28. By February 20 enough new growth had been made to provide a second set of

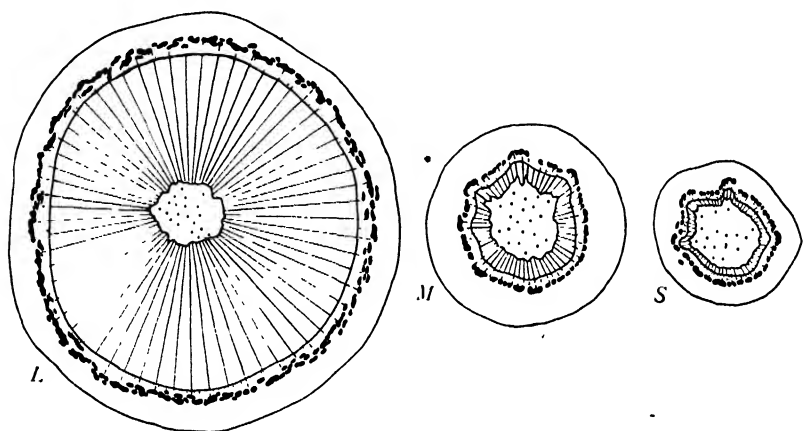


FIG. 8.—Transverse section of branch from each of three representative trees given different amounts of water, to show comparative topography of various tissues in three lots (camera lucida outlines); details of sections shown in figs. 9-11: left (lot L), large amount; center (lot M), moderate amount; right (lot S), small amount

samples. Examination of this sectioned material indicated uniformity in this young growth. The third set of samples, taken one month after the second (on March 21), again showed no outstanding difference between the four lots. As is normal in any tree growth, there was considerable variation within each lot, apparently as great as between the four lots. Even by that date the different watering had not made its effect noticeable. The fact that the soil had been thoroughly saturated at the time the trees were brought into the greenhouse probably accounts for this continued similarity in the four lots.

The fourth set of wood samples was taken from half the trees in

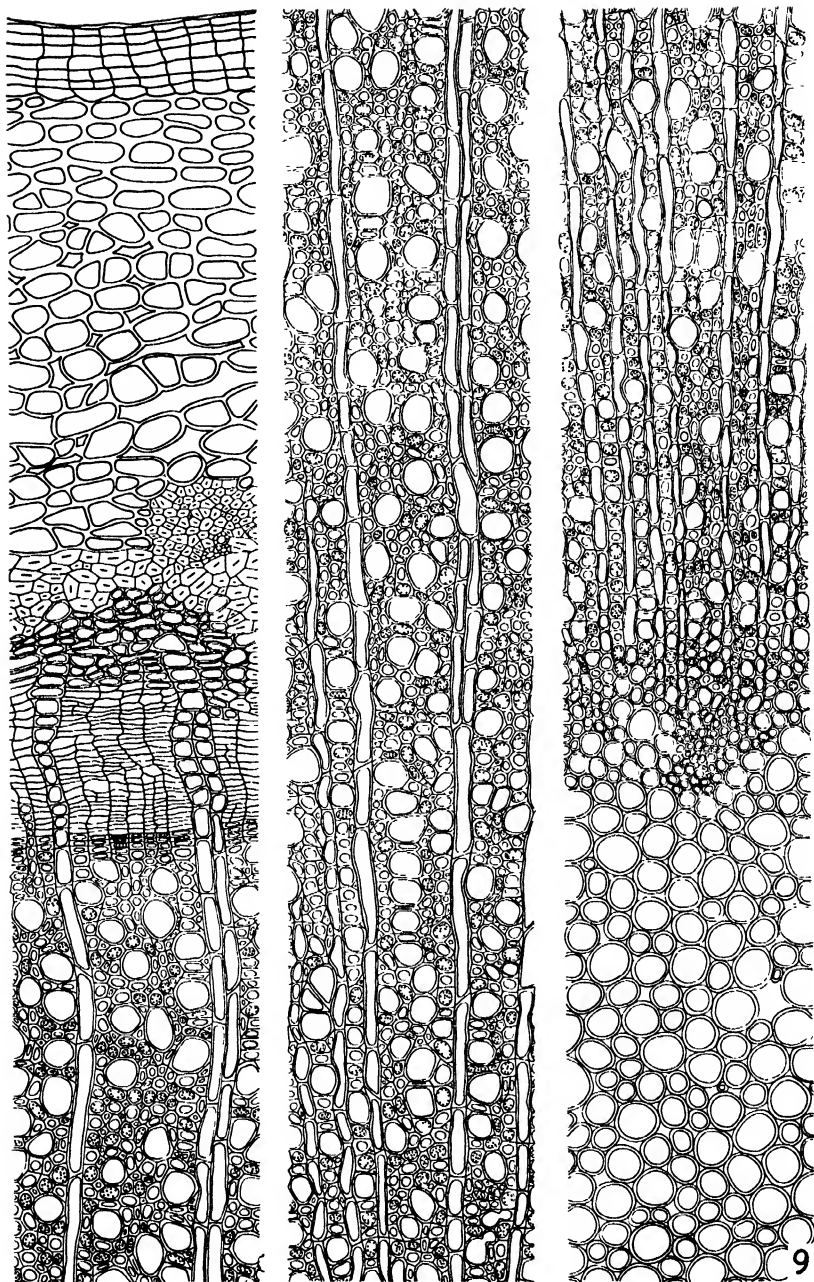
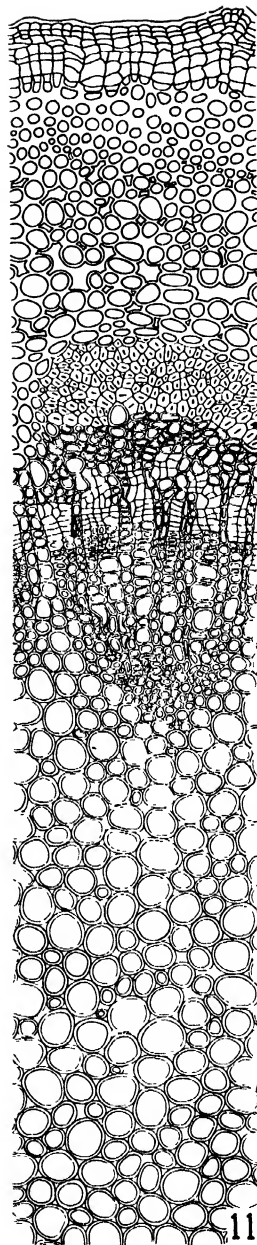
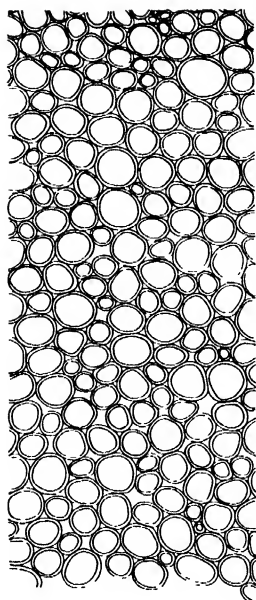
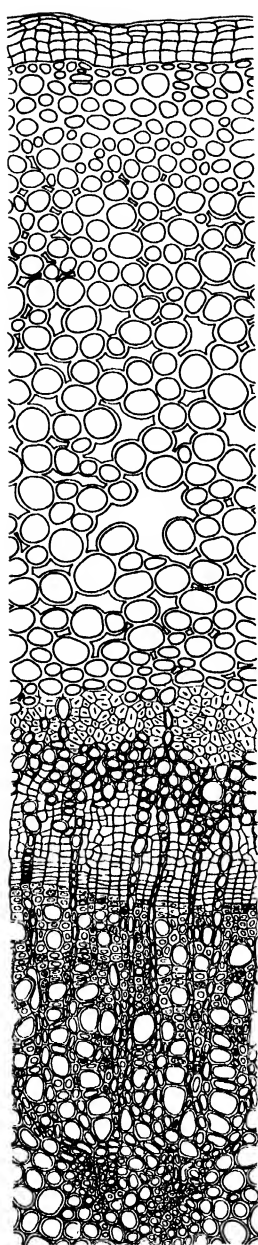


FIG. 9.—Detailed (camera lucida) drawing of narrow strip from cork to center of pith, from transverse section shown in fig. 8 L, taken from lot L (large amount); dotted cells in xylem represent parenchyma (scale same as for figs. 10, 11).



10

11

FIGS. 10, 11. —Detailed (camera lucida) drawings of narrow strips from cork to center of pith, from transverse sections shown in fig. 8 M and S (scale same as for fig. 9): left (lot M), moderate amount, cork through xylem; center (same), continued from xylem to center of pith; right (lot S), small amount, cork to center of pith.

each lot on June 16 and from the other half on July 1, or approximately three months after the previous samples had been removed. In these samples the first pronounced difference was found to hold consistently throughout a given lot of trees.

Comparing the trees in lot L with those of lot F, however, there does not seem to be any outstanding difference. Both lots show a range of variation, but not such that it is possible after a minute study of the sectioned material to say that any one thing is typical either of lot L or of lot F and is not to be found in the other. Because of this similarity, lot L only was finally selected for the detailed study of the heavily watered trees.

The fifth set of samples was taken December 27 while the trees were fully dormant, just before being started on their second season of growth under the conditions of this experiment. In this final collection for the year, one typical branch was taken from each lot. Figs. 9-11 are from this set, and bring out the contrasts between the trees in the heavily, medium, and scantily watered lots. The sample from lot L is from the first of two growths made in the one season from the one branch. The others represent but one growth.

In fig. 8 the cortex appears about the same in depth in all three lots, with the medium-watered lot showing somewhat the greatest depth. The pericyclic fibers, while more interspersed with parenchyma cells in lot L than in the other two, are well developed in all. The phloem seems not pronouncedly different in size in the three lots. The pith shows a marked uniformity in size in all lots. The xylem provides the most noticeable contrast in these contour drawings, where lot L shows a depth of xylem equal to the diameter of the entire section of the scantily watered sample. A numerical comparison of distance from cambium to pith in lots L, M, and S is 15 to 2 to 1 respectively, which if translated into terms of total area would show the heavily watered lot having an enormously greater development of xylem than either of the other two.

In the detailed drawings (figs. 9-11) of these identical cross-sections, additional facts are brought out which are shown in table II. Only the pronounced differences have been recorded. This table shows that the differences in the effect of varying water supply are not limited to the total or proportionate amount of the separate

TABLE II

CELLULAR DIFFERENCES ASSOCIATED WITH VARYING AMOUNTS OF WATER

Lot L, LARGE AMOUNT OF WATER	Lot M, MEDIUM AMOUNT OF WATER	Lot S, SMALL AMOUNT OF WATER
<i>Cork</i>		
Cells large	Cells medium	Cells small, most layers
<i>Cortex</i>		
Sclerenchymatous cells largest, oval in outline Parenchymatous area medium in size, smallest in rows of cells from cork to pericyclic fibers; cells large, oval	Sclerenchymatous cells medium, circular in outline Parenchymatous area large; largest in rows of cells from cork to pericyclic fibers; cells rather large, circular	Sclerenchymatous cells small, circular in outline Parenchymatous area small in size; slightly above L in rows of cells from cork to pericyclic fibers; cells small, circular
<i>Pericyclic fibers</i>		
In several bands interspersed with parenchyma cells Some cells large; some stone cells present	Similar to S in cell size and depth of area	Similar to M
<i>Phloem</i>		
Largest in area, largest in number and size of cells	Medium in area and in number and size of cells	Smallest in area and in number and size of cells
<i>Cambium</i>		
Cells largest	Similar in M and S	Similar in M and S
<i>Xylem</i>		
Enormous development; all cells largest; vessels most in number and largest in size. Ray cells longest and widest; least definite "summer" wood	Area much smaller than L, much larger than S; all cells smaller than L, slightly larger than S; largest and most definite area of "summer" wood	Area smallest in extent, cells fewest in number and smallest in size. Narrow band of definite "summer" wood. No xylem parenchyma cells other than rays
<i>Pith</i>		

Size of individual cells, thickness of cell walls, and total extent of pith area seems practically uniform in all lots

tissues, as shown in the topographical cross-section, but to the individual cells as well.

The heavily watered lot shows in its cellular composition a decided increase in the size of almost all cells, cortical, fiber, phloem, xylem; the vessels in the latter being much larger in the last-formed wood than those in the same position in the sections shown from lot M or lot S. The moderately watered lot, in the section illustrated, shows the greatest development of cortex, but in most other respects appears to take a medium position in both size and number of cells between lot L and lot S.

The scantily-watered lot has, in general, the smallest and fewest cells. It shows the greatest development of cork in number of layers, however, though not in actual depth of area. The underlying sclerenchyma seems proportionately heavier in this lot with smaller cells and thicker cell walls. All cortical cells in lots M and S appear circular in outline, whereas such cells in lot L are oval, with the longer axis in the tangential position. In phloem and xylem the cells are smaller in size and fewer in number than in the other lots.

The pith area in all lots appears to be practically uniform in size and number of individual cells, and in thickness of cell walls. This is the only feature in which there is noticeable similarity throughout, but here it is very pronounced.

The production of "summer" wood as compared with "spring" wood is most evident in lot M and least evident in lot L.

Discussion

From the data presented it is apparent that the different lots provide strong contrasts which are directly associated with the amount of water applied. In most of the gross characters, such as size of tree, number of branches, number of blossom clusters, size and number of leaves, etc., the results in general accord with the findings reported by a number of investigators, and so scarcely need reviewing in detail to emphasize the broader relation of moisture supply to plant growth. Certain other features, however, are worthy of special note.

The present experiment does not serve to illustrate the danger of over-irrigation mentioned by many writers, since even those trees

receiving the greatest amount of water, which under other conditions might prove detrimental, suffered no apparent injury from this source.

With regard to the two different systems of applying the same total amount of water, the close similarity of the results secured in lots L and F has made it impossible to conclude that either method is better than the other. The situation is probably somewhat analogous to that reported by VEIHMEYER (21), who states that provided the moisture content is above the wilting coefficient, roots apparently are able to obtain water as readily when the moisture content is low as when the soil is saturated to its maximum capacity. The fact that the soil in both lots L and F was never deficient in water may account for the uniformity of growth. The same would be true of the fruits in these two lots. With moisture present at all times considerably above the wilting coefficient, even though possibly fluctuating more in L than in F, it would seem that the fruits in both lots could develop at their maximum rate, so far as this was affected by water supply, regardless of whether that supply was maintained by a greater or lesser frequency of application.

This same situation would appear to account for the findings of LEWIS, KRAUS, and REES (15), who observed little difference in the experimental plots between 1000 and 3000 gallons per tree throughout the growing season; and for the experience of BATCHELOR (3), who found as large size and large yield of peaches with 31 acre-inches of irrigation as with 62 acre-inches under the same condition. If, as seems to be the case, the lesser amount was able to give maximum development so far as this was controllable by watering, doubling the supply would not be expected to increase the development. On the other hand, it would appear that in lot M, while reducing the seasonal amount of water to half that given the heavily watered trees evidently did not bring the water supply so low as to interfere with maximum fruit development in the year of its small crop; it did result in much smaller fruits in the year of its large crop, showing that trees of not greatly increased size may require much larger quantities of water to develop a large amount of fruit.

The results in lot M would also seem strongly to support the evidence of other investigators in favor of thinning of fruits to secure increased size in case of water shortage. In one experiment (15) it

was found that several check (unirrigated) trees when carrying a very light load produced apples which would compare favorably with those from the irrigated plots; but of another orchard it was stated, "if at least one-fourth more of the fruit had been removed at thinning time, there would likely have been not only a much larger percentage of commercial fruit but more boxes of first-class apples."

Referring to lot S, which matured no fruits the first blossoming year, and only three fruits the second year (fig. 7), this poor set as well as the small size of the fruits which did mature seem directly attributable to the water supply, either alone or as affecting the metabolism of the trees. HEINICKE (11) states that unfavorable conditions of nutrition and water supply are two basic factors causing the normal drop of flowers and partly developed fruits of the apple. CHANDLER (6) mentions that great water deficit, whether resulting from high transpiration or from limited water supply, may cause abscission of nearly all fruits. He further adds that if the water supply should be inadequate through a considerable part of the day, the leaves with their much greater osmotic concentration might pull water away from the fruits. This, under conditions of moderate shortage of water, may account for the smaller fruits.

The poor quality of the S fruits, and of the M fruits in the year of the large crop, would appear to be explained by SORAUER'S (20) statement that with a scarcity of water there is less increase in stored food materials: acid is less, sugar is less, giving an insipid taste and poor quality.

As to the gross histological differences brought out in fig. 8, these show that the most noticeable effect associated with increased water supply is the greater depth of the xylem area. This would accord with the findings of HUNTINGTON (12, 13), DOUGLASS (7, 8, 9), MACDOUGAL (16), ANTEVS (1), and others making a study of the annual rings of forest trees in relation to climate, who report that with limited moisture, rainfall was unquestionably the controlling factor in growth as shown by width of ring. But in moist climates the trees seemed to depend on other elements, such as temperature or a combination of elements.

PEARSON (18), studying saplings of western yellow pine, found moisture the dominant factor, the April and May precipitation being most important in determining the amount of height growth secured.

That there was an extended period of growth associated with an increase in water in the present experiment is shown by the delay in forming terminal buds on the shoots in lot M as compared with lot S, and the much longer growing period of lot L, even to the extent of second and third growths in the one season. These observations emphasize the importance of moisture supply in connection with the laying down of new wood, when other nutrients are not lacking.

With regard to the microscopic differences as shown in table II and in figs. 9-11, the contrast in development is seen to be most pronounced. The evidence presented does not agree with that reported by CANNON (4), however, who states that the non-irrigated stems of the desert plants studied have larger ducts and more of them per equivalent cross-section than the irrigated stems. He notes, however, that VOLKENS, studying Egyptian desert plants, found contrary results, namely, that the ducts of the stems of the shrubs and trees were relatively poorly developed, which latter would appear to confirm the present evidence. Here it is shown for the pear, and seen to some extent in lot M as compared with lot S, that associated with additional water is an increase, especially in the number and size of certain types of cells, including vessels, an increase which in lot L amounts to a great development of conducting area as compared with that in the other two lots. Instead of a small amount of water in lot S causing small cells with thick walls to be formed, as PALLADIN (17) mentions, in the pear at least far fewer cells are produced under a shortage of water. Further, the cells formed are not provided with thicker walls, even though compared with the smaller size of cell they may appear so. It would seem that reducing the water supply to a minimum has not caused the plant merely to produce a given quota of cells which are smaller and thicker walled than might have been secured by providing more water, but rather, the shortage of water has resulted in stopping all cell division and enlargement beyond that which followed the first heavy watering of the year.

There is an additional point which might be of considerable practical significance to men interested in forestry, as well as to fruit growers and nurserymen. While there are instances perhaps where this is not true, in the case of the pear tree at least the great develop-

ment of xylem, accompanying increased water supply to the trees in rich soil, apparently may result not in an expanded zone of spongy, succulent tissue, but in a greatly increased volume of firm, sound wood.

The uniformity of pith in all lots is in accord with the expectations from a knowledge of plant growth. In the pear the pith is entirely a primary structure. The cells, upon being differentiated at the growing point, mature early, after which further growth ceases. In this instance, to start growth at the beginning of the season all trees were given a uniformly heavy watering. The effect of this would naturally be reflected in the primary tissues, giving uniform early growth. The reduced water supply later would appear to have made its effect evident first in the early stopping of length growth, which would result from no more cells being differentiated at the growing point. The later effect would be evident in the secondary meristem and result in reduced and finally stopped growth from the cambium.

In an attempt to account for all the gross differences noted, the results would seem to suggest a number of possible interpretations.

Recent investigators have shown the importance of the individual plant or even a part of the plant and its nutritional balance at any particular time as correlated with the type of response which may result from the treatment accorded it, whether pruning, fertilizing, shading, ringing, defoliating or what not. There is in this, then, one possible explanation for the results of the present experiment, and that is that the varied water supply, coupled with an abundant nutrient supply, modified the nutritive conditions within the trees.

In some respects there appears to be a somewhat close similarity in these four lots of trees to those of the four classes or groups suggested by KRAUS and KRAYBILL (14). Under the conditions of the experiment, the trees in lots L and F, which were given an abundance of water, gave a highly vegetative response both in external and internal characters. Also, while these trees when considered as a whole did produce blossoms and did mature fruit, such were frequently limited to certain parts of the tree, while many of the branches which made long growths failed to flower. These trees would seem to correspond to the class II trees but be closely ap-

proaching those of class III. The trees given a moderate supply of water, lot M, and those given a scanty supply, lot S, would seem to accord rather definitely with class III and class IV trees respectively.

This close similarity is of more than passing interest. At the same time there are insufficient data available to permit of more than the mere recording of the general similarity and noting its possible bearing in interpretation of the results secured. It would appear that under the conditions of this experiment a change in the amount of available soil moisture definitely caused changed expressions in tree growth, somewhat similar to those secured by other workers through changes in the nutritive balance brought about by various practices other than alterations in the water supply. Another possible explanation for the results secured is that the effect may have been largely a physical one, or a combined physical and chemical one.

Knowledge of plant growth emphasizes the importance of turgor in cell division and enlargement. The presence of an abundance of water would be expected to provide a somewhat constant condition of turgidity, and hence, other things being equal, a condition favorable for growth. Decrease of the water supply to a minimum would probably so reduce cell turgor in the meristematic regions that finally further active growth, even if nutritionally possible, would be physically impossible.

Evidently the amount of water which a tree uses does have a definite effect on the metabolic processes as shown by different expressions of growth. Where growth is checked accompanying a reduced water supply, this may be due to a single factor or to a combination of factors. It may be caused by a limiting of the supply of nitrogen, as some hold, or to an accumulation of carbohydrates. CHANDLER (6), however, contends that there "is no convincing evidence that the mere accumulation of carbohydrates inhibits growth though it might cause the nitrogen to be combined in larger organic molecules and possibly in forms less active in immediate growth." Again, the cause may be a limiting of both nitrogen and carbohydrate content, or it may be the result of alterations in the metabolism induced by lack of sufficient water to expand the tissues, the loss of turgidity being responsible for retardation and finally cessation of

development. In the present instance there is not sufficient evidence to establish proof one way or another.

It is not intended to give the impression that water of itself can bring about maximum plant development. The great importance of other factors as affecting the metabolic processes must not be lost sight of. In this connection, the relation which both the physical condition of the soil and the supply of available nutrients may bear to the water requirement of plants is of special significance.

GARDNER, BRADFORD, and HOOKER (10) state:

Few realize that, when the soil provides conditions for tree growth that are optimum from the standpoint of nutrient supply, actually less water is required for a given yield than when the plant is handicapped because of the lack of some nutrient as well.

They also refer to LEATHER, who studying the question in India reported that

the effect of a suitable manure in aiding the plant to economize water is the most important factor which has yet been noticed in relation to transpiration.

They also add that

the reduction of the water requirement of the plant by maintaining the soil in a condition as near as possible to the optimum with respect to nutrient supply should be a constant and conscious aim in scientific orchard management.

This correlation between available plant food and water requirement is further emphasized by POWERS (19), working with field crops, who states

frequently in our experiments application of a simple fertilizer has saved from a quarter to a half of the total irrigation and has doubled the returns from each unit of water.

He also reports that at times one ton of manure may equal 100 tons of water in securing returns. In an experiment with fruit trees (15) still further evidence is presented, where trees on good soil with cultivation but no irrigation were found to produce not only more but larger fruits than those on poorer soil which had an abundance of water. This serves to show not only that favorable nutritive conditions make for water economy, but that irrigation cannot be expected to make up for poor quality of soil.

Summary

1. The experiment here reported was undertaken to determine the effect of varying amounts of water upon both the gross and the microscopical development of *Pyrus communis*.

2. Forty-eight dwarf pear trees were divided into four lots, all lots being treated uniformly in all respects save for watering.

3. In the two heavily watered lots, applying the same total amount of water, but in more frequent applications of smaller quantities at a time, appeared to give results practically identical with larger applications at less frequent intervals.

4. Flower clusters were more abundant on trees given more than the minimum amount of water. The number varied between moderately and heavily watered trees, depending in part on the crop of the previous season. The number of flowers to each cluster averaged less when abundant water was supplied, due to the high percentage of axillary clusters produced, as compared with less heavily watered trees which had a high percentage of spur clusters, which latter produced proportionately more flowers to each cluster.

5. Abscission of blossoms and partly developed fruits was more pronounced where least water was available. Two types of abscission were noticed between the trees given least water and the others.

6. The number of fruits matured by a tree in any one season appeared to have a greater effect on the size of the individual fruits and on the crop produced the following year than did the actual amount of water applied to that tree. With a small crop of fruit, moderate watering was associated with production of full-sized, normal fruit. With a large crop, moderate watering was associated with production of much smaller and a poorer quality of fruit than when abundant water was applied. A pronounced shortage of water resulted in small fruits of poor flavor and quality. The value of thinning to secure increased size in case of water shortage is emphasized.

7. Abundance of water was accompanied by increase in all types of vegetative growth and in length of the growing season; scarcity of water by lowest vegetative responses; a moderate supply of water by a medium vegetative growth.

8. There appeared to be a close qualitative and quantitative relationship between shoot growth and fruit development, regardless of the amount of water that a tree received. A large fruit crop seemed to make less demand on the water supply than did large shoot growth.

9. A detailed histological study showed the most pronounced difference in gross topography of the tissues to be in the extent of the xylem area. As between a large, medium, and small amount of water, this stands in the proportion of 15 to 2 to 1 respectively. The cortex is largest in the moderately watered lot.

10. A comparison of cells showed a number of definite differences in the cortex, phloem, and xylem cells in the different lots, especially as to number and size. The pith cells seemed practically identical in all lots, regardless of the amount of water applied.

11. In the pear at least, a great development of xylem may accompany increased water supply to the trees in rich soil without resulting in succulence, but in a greatly increased volume of firm, sound wood.

12. An attempted interpretation of the results secured suggests that, following changes in the water supply, alteration in the metabolic processes may be due to physical or nutritional causes or both.

13. Emphasis is laid on the relation which the physical condition of the soil and the supply of available nutrients may bear to water requirement in securing its greatest practical efficiency.

The present delayed study of the sectioned material, prepared by the writer while connected with the Oregon State Agricultural College, has been made possible through the courtesy of Professor W. S. BROWN and DR. E. M. HARVEY of that Institution. DR. E. J. KRAUS of the Department of Botany of the University of Chicago gave valuable suggestions and criticisms during the investigation.

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CYTOLOGICAL STUDY OF OEDOGONIUM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 407

HIRO OHASHI

(WITH PLATES I-III AND TWENTY-ONE FIGURES)

Most algae as common and as important as *Oedogonium* have been studied ever since the first microscopes were made; but while the morphology and taxonomy of this genus have been investigated by various workers, little information has been obtained concerning its cytology. Consequently it seemed worth while to make a cytological investigation of *Oedogonium*, with modern methods of technique.

Material and methods

Material used for this study, consisting of four species, was collected from four different places at various times. One new species (*O. nebraskense* Ohashi) (12) was collected by Dr. ELDA WALKER on April 22, 1919, in small ponds at Lincoln, Nebraska, and turned over to the writer. The second one, *O. americanum* Transeau, was given by Dr. G. S. BRYAN, who collected it in the vicinity of Madison, Wisconsin. The third species, *O. grande* Kuetzing Wittrock, was gathered in 1924 from a small swamp at Palos Park in the vicinity of Chicago. The fourth was an unidentified species from lagoons in Jackson Park, Chicago, collected in 1926.

In 1924 the collection of material was begun on July 3 and continued at frequent intervals until September 10; and in 1926 it was begun on May 17 and ended August 15. In both years collections were made several times at each place. Some of each collection was fixed immediately, but the greater part was kept growing in jars in a window of the laboratory.

Throughout the investigation, living material was studied both in the field and in the jars kept in the laboratory. When collections were abundant, some of the material was kept in water from the pond, and some was grown in various nutrient solutions which might vary the course of the life history.

The material collected by Dr. WALKER was fixed and preserved in a chrom-alum solution (water 100 cc., chrom alum 2 gm., formalin 1 cc.), but the remainder was fixed in chrom-acetic acid (water 100 cc., chromic acid 1 gm., acetic acid 3 cc.), with ten drops of osmic acid (1 per cent) to 100 cc. of the solution.

The zoospores and the sporelings were wrapped in onion epidermis, which was killed with alcohol and washed with water; and then were fixed and stained in the same manner. This method was suggested by Dr. KUSANO of the Tokyo Imperial University. In using the paraffin method, when the material was cleared with xylol the filaments were put together as straight as possible, and then were kept under a light slide in a petri dish. During infiltration with melted paraffin, the material was kept also under a heavy cover glass in the dish.

Most of the material was stained with Haidenhain's iron-alum haematoxylin, but some with Magdala red and anilin blue; and mounted in Venetian turpentine. Paraffin sections were cut 5 or 6 μ thick and stained with Haidenhain's iron-alum haematoxylin.

CELL DIVISION

For this purpose, *O. grande*, collected from Palos Park, was used. Throughout this account the correlation between the somatic mitosis and the development of the ring will be traced, but first a brief sketch is given of the resting nucleus of the vegetative cell to contrast with the various changes during the division.

RESTING NUCLEUS.—The vegetative cells are cylindrical, containing peripheral reticulate chromatophores in which many pyrenoids and minute starch grains are scattered. The very small starch grains have been described as the first visible product of photosynthesis. Each pyrenoid is also surrounded by somewhat larger starch grains derived directly from the pyrenoid. The resting nucleus is generally circular and flattened in shape, and lies in the peripheral protoplasm a little more deeply placed than the chromatophore, and at the same distance from both cross walls of the cell (fig. 1). The nucleus has a distinct nuclear membrane, within which is a very fine reticulum. At the nodes of the reticulum there is an abundance of chromatin; and there are one or more nucleoli (fig. 2).

MIGRATION OF NUCLEUS AND APPEARANCE OF RING.—The first indication of cell division can be observed in the migration of the nucleus toward the upper end of the cell, until it reaches a point about two-thirds of the distance from the base to the top of the cell (fig. 3). Then the nucleus begins to enlarge, usually changing its shape from circular to ellipsoidal or spindle-shaped, but without any particular change in its structure. While the elongated nucleus is still in the same condition, a very narrow ring appears along the longitudinal cell wall at the extreme upper end of the cell. First it looks something like a streak connected with a tiny elevation originating from the second layer of the cell wall (fig. 4); but as it develops one can easily find a fine circular splitting on the surface of the ring. The free edges of the ring at the splitting are in connection with the second layer where the elevation has appeared.

PROPHASE.—The first step of the prophase begins by the breaking down of the reticulum, showing some lighter areas at certain regions; and is followed by the appearance of the very irregular zigzag threads of chromatin and reticulum of uneven thickness everywhere in the nucleus (fig. 5). Uniting with each other, denser masses of chromatin begin to condense into the chromatic threads of even thickness, while the lighter spaces become wider and clearer by the disappearance of the reticulum (figs. 6, 7). Gradually these threads begin to straighten, and finally form a single slender and coiled chromosome thread, the so-called spireme. At this stage the nuclear membrane has disappeared.

The longitudinal splitting of the chromosomes appears in these slender threads, beginning as small vacuoles along the axis, but later developing a more or less continuous split resulting in two parallel strands (fig. 8). These strands become shorter and thicker and finally are segmented into a definite number of chromosomes. At this stage the longitudinal split becomes obscured by the close association of the halves, which, however, still can be seen (fig. 9). While these chromosomes are at their highest contraction the spindle is formed in the cytoplasm, consisting of fine fibers and numerous minute granules.

RING IN PROPHASE.—During the nuclear prophase the ring grows rather rapidly, increasing its diameter to five or six times that of the

earliest stage; and also its wall becomes thicker, showing obscurely two layers. The split of the ring increases its width, and it becomes clearly visible at the point of attachment to the second layer of the cell wall.

METAPHASE.—The chromosomes now lie close together at the equator of the spindle, forming the so-called equatorial plate. At this stage they show a great diversity in their forms and sizes. The forms may be classified into three main groups: short rod, long rod, and loop; while the sizes are entirely different. It is not difficult to trace the longitudinal splitting along the axis in some chromosomes (fig. 10). The spindle attachments of the chromosomes are correlated with their various differences in shape. These different attachments may be grouped into terminal, subterminal, median, and submedian, according to WILSON's classification (fig. 11).

ANAPHASE.—Each double chromosome begins to separate at the place where the fibers are attached, and the two parts gradually move from the equatorial plate (fig. 12). Then both groups become entirely free and proceed to opposite poles of the spindle, showing the characteristic forms of the individual chromosomes (figs. 13, 14). As soon as these daughter chromosomes reach the poles they begin to contract into dense masses, in which the individual chromosomes can still be distinguished in some degree (fig. 15).

RING IN METAPHASE AND ANAPHASE.—Although it is difficult to name any particular stages in growth during these periods, the ring continues its growth in the same way as in the preceding period; that is, at the end of the anaphase it increases its diameter to about seven or eight times that of the first appearance, and there is a thickening in both layers of its wall. The portions of the free edges of the ring where they are in contact with the cell wall become so evident that one can observe them without difficulty. The ring seems to have some kind of mucilage in its cavity which stains dark with iron-alum haematoxylin.

TELOPHASE.—After remaining some time closely crowded together at the extreme ends of the spindle, the chromosomes of each daughter nucleus begin to separate from one another to reconstruct the resting nucleus. At the same time their boundaries become scarcely visible, and within each chromosome minute vacuoles arise

along its axis (fig. 16). While these vacuoles are developing the volume of the nucleus increases, but it is still smaller than the ordinary resting nucleus.

Both daughter nuclei now approach each other at the equator of the spindle (fig. 17), and when they come almost into contact, numerous fine granules appear around them. These fine granules gather together, forming a thin wall between the nuclei. At this stage in the nucleus a more and more uniform reticulum is found, in which the nucleolus begins to appear, while the limits of the disappearing chromosomes still can be recognized (fig. 18).

The nuclear membrane seems to make its appearance during this stage. After formation of the separating wall the daughter nuclei move apart and take their position at the center of the peripheral protoplasm. The nucleus shows all structures of the resting condition (fig. 19).

RING IN TELOPHASE.—At the end of this stage the ring probably reaches its full growth, increasing $1-2\mu$ in diameter, and there is also some increase in thickness. The opening of the split in the ring becomes wider and very conspicuous.

DEVELOPMENT OF RING INTO NEW CELL WALL.—After remaining for a while in the same position the upper nucleus moves near the ring, which then begins to stretch. Between the stretching ring and the outer layer, the mucilaginous mass is sometimes observed (fig. 20). The portion of the outer layer which was associated with the stretching ring begins to break up, leaving zigzag lines at both edges (fig. 21). The stretching is followed by a straightening, probably caused by the turgidity of the cell contents.

At the same time the separation wall between the new cells moves up and unites with the inner layer of the cell wall at the joining of the new and the old wall (fig. 22). The layers shown in the wall of the ring now become the outer and inner layers of the new cell wall, although they are still very thin. This thin new cell wall continues its growth until it reaches the usual length and thickness.

SPERMATOGENESIS

Antheridia are borne in two kinds of filaments. In macrandrous species they are borne in the same filament with the oogonia, or in

dioecious species in filaments of about the same size as the female filaments. In nannandrous species the male filaments are so small that they are called dwarf males, and they are epiphytic on the female filaments, usually on the suffultory cell. The formation of antheridia is so different in the two types that they will be described separately.

MACRANDROUS TYPE

In this type there are two methods of forming antheridia. In one, *O. grande*, the antheridia are formed in a long series which may consist of more than thirty antheridial cells occurring at one or more places in a filament. In the other there is a short series consisting of a few cells arising successively. The essential features in the formation of antheridia in the two cases are sufficiently similar to be described together, however, except in the mode of formation of sperms.

FORMATION OF ANTHERIDIA.—At the beginning of the formation, the antheridia of *O. grande* show the same migration of the nucleus toward the upper end of the cell which was observed in vegetative cell division. In this case, however, the nucleus almost reaches the cross wall. Then a small ring makes its appearance at the same place; but it does not grow as much as in vegetative cell division. The mitosis now takes place just as in somatic division (fig. 23). As a consequence of this division there is formed a small antheridial cell at the upper part. This process is repeated by the larger antheridial mother cell (fig. 24).

CELL DIVISION IN ANTHERIDIA.—After the antheridial cells reach a certain number, each of them begins to divide into the two sperm mother cells. During this division all important phases of the mitosis can be seen without difficulty (figs. 23–26). The most striking feature in this is that the figure is not oriented along the vertical but along the horizontal axis of the cell, probably because the cell is so short in proportion to its length. The result of this arrangement is that at first the two daughter nuclei lie side by side (fig. 25*d*), although the cell is to be divided transversely; so the stretching of the ring begins before the formation of the separation wall (figs. 25, 26). This feature is also different from the somatic mitosis. As soon as the ring has stretched, one of the daughter nuclei moves upward,

where there is more room (figs. 25, 26), and then a thin wall begins to appear between the nuclei (fig. 26*w*). In this way the sperm mother cell is formed.

FORMATION OF SPERMS.—The sperm mother cells start to form the two sperms in each cell, lying side by side. So far as the mitosis is concerned, this cell division follows the same method observed in the antheridial cell, although it is too small to show details (figs. 27, 28). But the distinguishing differences are that there is no formation of a ring, and also that the sperms are developed from the two daughter cells. After the formation of cilia is completed the swimming sperms escape from the cells (fig. 29).

NANNANDROUS (DWARF MALE) TYPE

ANDROSPORANGIUM.—Preceding the formation of the dwarf male, the androsporangium appears in the female filament at a short distance above the oogonium in *O. nebraskense* (12). It is formed by a typical vegetative *Oedogonium* cell division, but is much smaller than the vegetative cell. There may be a single androsporangium, or two, or sometimes three or four in a series. When the androspores are mature they swim about for a while, and then attach themselves to the suffultory cell where they develop into the dwarf males (fig. 30).

DWARF MALE.—The androspore forms first a basal vegetative cell, the so-called stipe (fig. 31), which gives rise to a ring at the top of the cell (fig. 32). The nucleus then divides as usual (fig. 33), and by stretching of the ring the first antheridial cell is formed at the top of the stipe with a cap at its head (figs. 34, 35). After the nucleus in the antheridial cell has remained in the resting stage for a time (fig. 36) it begins to form the two sperms, separated transversely by a thin cross wall (figs. 37, 38). Then a second ring arises in the stipe near the place of formation of the first one (fig. 39). Sometimes it appears before the first antheridium has given rise to the sperms. After nuclear division the second antheridial cell is formed just beneath the first one (fig. 40). The nucleus in the second antheridial cell divides into two and another pair of sperms is discharged (fig. 41).

At the beginning of the formation of the sperms usually the new

daughter cells begin to contract in the antheridial cell. The nucleus takes a position at some corner of the cell, leaving a clear space at the opposite side, in which something like the blepharoplast appears first as a circle of granules just under the plasma membrane. Each granule then seems to give rise to a cilium. The antheridial cell of the dwarf male being much longer than that of the macrandrous type, development of the cilia can be recognized with comparatively less difficulty (figs. 39-43). While the second antheridium is still forming the sperms the first is generally discharging them.

OÖGENESIS AND FERTILIZATION

OÖGENESIS

The oogonium may develop from any of the vegetative cells, but most frequently it arises from a cell well supplied with chlorophyll, starch grains, and other food substances, and is called the mother cell. There are two types of mother cells; the one having characteristic swellings of its longitudinal cell wall (as in *O. nebraskense*) is easy to recognize (fig. 44), while in the other type (*O. americanum*), apparently without any such modification, it is rather hard to distinguish whether it is going to be a mother cell or an ordinary vegetative one. If more attention is paid to the cells, however, there may be found some differences between them.

In the first place, the mother cell is usually longer than the ordinary vegetative cell; and also, when the vegetative cell divides, the nucleus generally migrates toward the upper extremity of the cell; and as soon as the ring begins to form the nucleus passes into the mitotic process. But in the mother cell, even after the formation of the ring, the nucleus not only remains in the resting stage for a rather long time but it stays at the center of the cell (fig. 44). Besides these features the contents of the mother cell in general are much denser than those of the vegetative cell.

This nuclear situation is very evident in *O. nebraskense*. In this species, when the ring attains its full growth the nucleus moves slightly in the opposite direction (fig. 45). The reasons the behavior is different from that in the vegetative cell division may be partly physiological, because the nucleus has to keep its activity longer for manufacturing food substances for the young egg; and partly mor-

phological, because the nucleus has to stay where the cell division takes place.

In oogenesis the ring naturally becomes more conspicuous than in spermatogenesis, because the oogonium is larger than the antheridium (fig. 46). While the cross wall is being formed between the two daughter nuclei the ring begins to stretch, and as soon as the wall of the oogonium is formed by the rupture of the ring, the greater part of the contents of the mother cell moves into the oogonium, accompanying the cross wall to the joining point of the new and old cell walls, and leaving large vacuoles behind in the old cell, which is now called the suffultory cell (figs. 47-52). When the oogonia are formed, the second one is always produced by the suffultory cell, forming the second ring just beneath the first oogonium (figs. 53, 54). After attaining a certain size, a pore or split is formed at some part of the oogonium wall; and the contents of the oogonium begin to constrict and round off, forming a single oosphere (fig. 52).

It is difficult to observe the nuclear behavior of the mitotic process, since the contents of the mother cell are so dense, but probably it acts just as in the vegetative cell division.

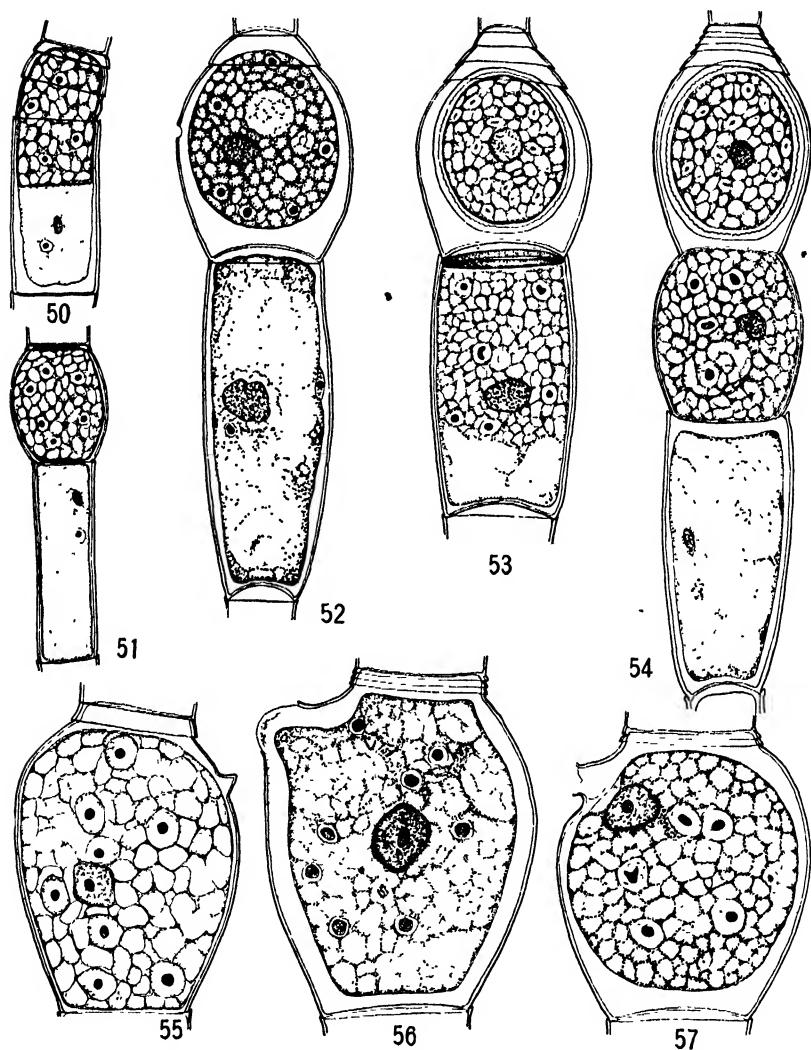
FERTILIZATION

Fertilization has been so well described by previous investigators, especially KLEBAHN (8), that an account of it need not be repeated here; but brief mention is made of what has been observed in *O. americanum*.

When the oosphere is ready for fertilization, it forms a pore at a certain point on the oogonium wall (figs. 55-57). The nucleus of the oosphere, which is in the resting stage, approaches its surface, facing the opening in the oogonium wall (fig. 57), which the swimming sperm then enters. In some species there seems to be nothing to hinder the sperm from entering the oogonium, because of the large opening (fig. 58). Then the sperm penetrates the oosphere by pushing the cell contents inward (figs. 58, 59).

Fusion of the two nuclei now takes place. The male nucleus is always smaller than the female and has only chromatin granules in its reticulum; while the female nucleus has in addition a distinct nucleolus. With both nuclei in the resting stage, a fusion of all the

nuclear material takes place (figs. 59, 60). As the process of fusion advances, both nuclear membranes, which have been in contact with each other, show in their final stages some irregular remains, out-



FIGS. 50-57. - Figs. 50, 51, various stages of oogenesis without suffultory cell; fig. 52, formation of pore and oosphere; fig. 53, second ring in suffultory cell; fig. 54, formation of second oogonium; figs. 55, 56, pore at upper part of oogonium cell wall; fig. 57, migration of nucleus toward pore.

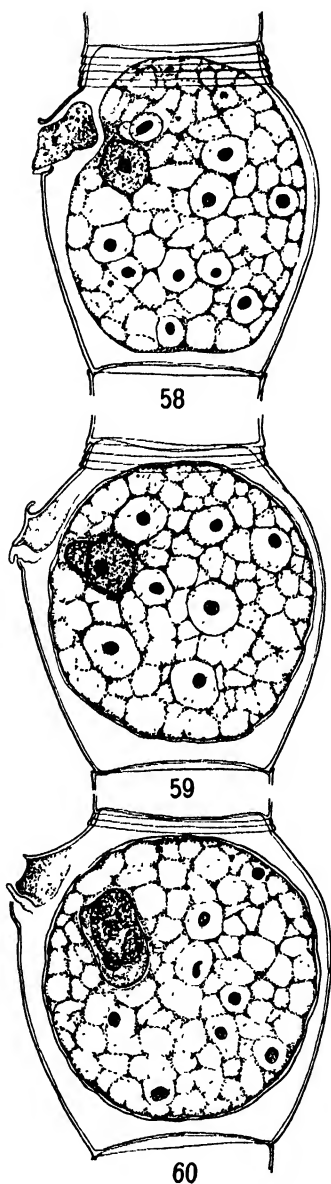
lining the form of the fusing nuclei (figs. 60, 61), which then begin to return to the center of the fertilized egg. When fusion is completed the nucleus generally shows a circular outline.

FORMATION OF SPORE COAT.—After penetration of the sperm into the egg the spore coat begins to develop. The first indication of its formation is the appearance of the numerous granules around the egg under the thin outer layer (fig. 61). Next the second wavy layer is formed of a certain thickness, the wide, smooth, inner part being added by deposition from the contents of the egg (figs. 62, 63). Finally the third or inner layer, which is rather smooth and thin, also arises from the granules deposited by the fertilized egg (fig. 64).

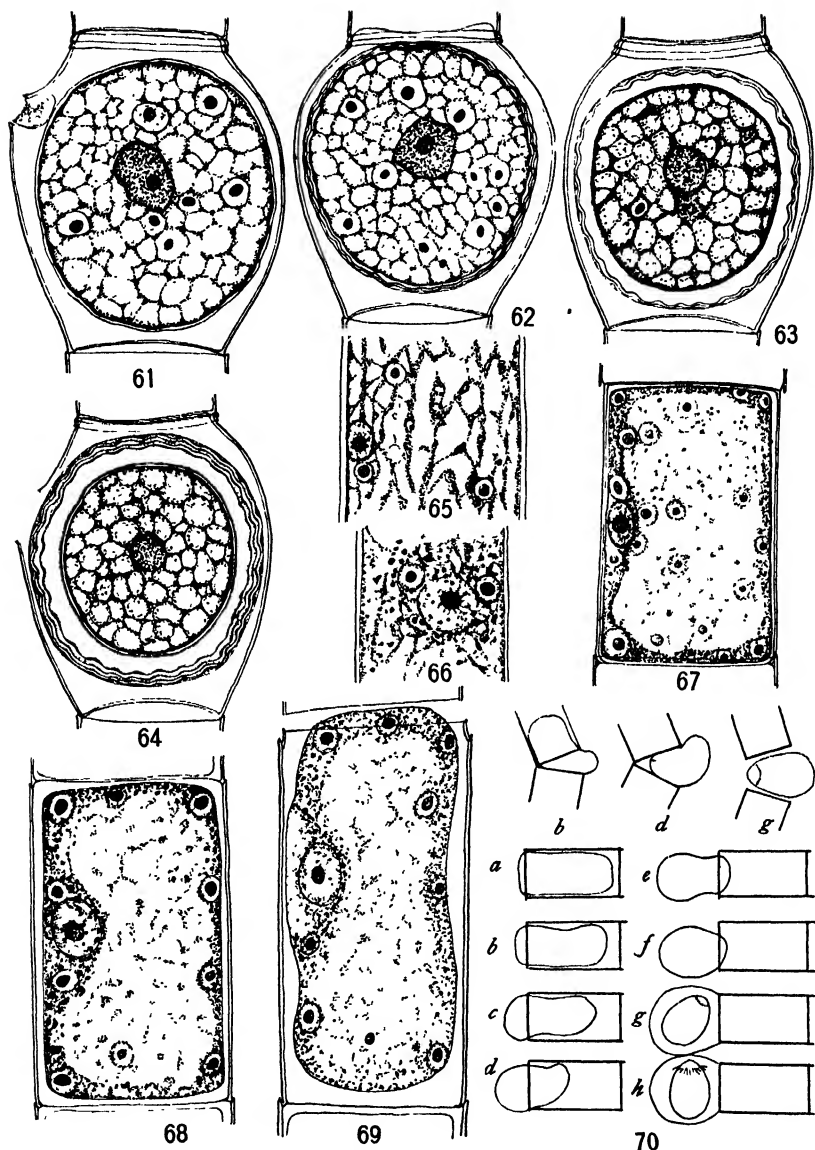
During formation of the middle and inner layers the outer layer increases in thickness. It is interesting to compare the thickness of the cell wall and the diameter of the fertilized egg, for the more the thickness of the cell wall increases the more the diameter of the egg decreases.

ZOOSPORE

Asexual reproduction takes place by means of zoospores which arise singly from any of the ordinary vegetative cells. The cell which is to produce a zoospore contains such abundant contents that the cavities between the reticulate chromatophores



FIGS. 58-60.—Fig. 58, entrance of sperm into oögonium; figs. 59, 60 fusion of male and female nuclei in resting stage.



FIGS. 61-70.—Fig. 61, fusing nuclei returning to center of egg, and appearance of numerous granules around egg for formation of spore coat; fig. 62, formation of wavy part of second layer of spore coat under thin first layer; fig. 63, formation of smooth part of second layer; fig. 64, formation of third inner smooth layer; fig. 65, position of nucleus in vegetative cell; fig. 66, sinking of nucleus beneath clear membrane; fig. 67, appearance of granules of different sizes around nucleus; fig. 68, nucleus deeply placed under clear space, with granules gathering along margin; fig. 69, splitting of cell wall for escape of zoospore; fig. 70, topographic figures showing escape of zoospore from cell.

begin to disappear, and the peripheral protoplasm becomes much thicker, decreasing the size of the large central vacuole.

The first difference recognized in the nucleus is a change in its position. In the ordinary vegetative cell it is in the peripheral protoplasm a little more deeply placed than the chromatophore (fig. 65); but now it moves inward, leaving a circular light area beneath the clear membrane (fig. 66). During this process the nucleus increases in size, but does not show any particular change in its constitution. Granules of different sizes begin to appear around the nucleus and gradually increase in number (fig. 67). Finally the fine granules gather much more densely along the margin of the circle (fig. 68); and it seems probable that the blepharoplast may arise from these granules, although this is still to be proved.

ESCAPE OF ZOOSPORE.—It is interesting to watch the zoospore escaping from the cell. Just before it does so there appears an active Brownian movement of some small particles in the protoplasm at the upper end of the cell, where the splitting of the cell wall is going to take place (fig. 69). As soon as the cell wall splits transversely, one end of the mass of cell contents begins to emerge from the slit, and at the same time the other end begins to round off, separating from the cross wall of the cell (fig. 70*b*). Gradually the whole mass escapes from the cell and shows an amoeboid movement (fig. 70*c-e*). When the whole body has emerged it begins to round off, and assumes the characteristic pyriform shape of the zoospore (fig. 70*d-f*), which at first is surrounded by a delicate hyaline bladder. While it is still in the bladder the numerous granular cilia become visible in a circle, at the colorless narrower end (fig. 70*g*), and then suddenly the cilia are spread out in a radiating arrangement (fig. 70*g, h*). The position of the cilia is always at a right angle to the longitudinal axis of the old cell. Then the zoospore begins to swim quickly. The entire process lasts only a few minutes, but the swimming stage lasts probably about one or two hours. This process was observed in the unknown species.

HABIT OF ZOOSPORE.—There may be several factors to stimulate formation of zoospores, but a change in temperature seems to be most effective. Whenever material was brought either from a warm to a colder place, or from a cold to a warmer place, numerous zoo-

spores appeared in two or three days. In August, for instance, after keeping material in the refrigerator for three days it was put on the window sill. Then the zoospores began to swim from early morning until late afternoon for about two days. Some material, which had been kept in Knop's solution for some time, was put into distilled water for two days and the same experiment repeated. At that time the formation of zoospores was so abundant that almost all filaments were broken into small pieces, after three days, by producing the numerous zoospores. Another example was noted on November 25, 1924. It was so cold that all jars of *Oedogonium* placed on the outside of the window began to freeze; so they were taken into the warm room. After five days each jar showed a remarkable number of zoospores. Whenever the material was put in Knop's solution or taken from it to distilled water, some zoospores were formed within two or three days. The same thing happened when the material collected from the field was washed with water to take off the dirt. While the zoospores are swimming they show heliotropism distinctly, and rest themselves at the surface of the water along the jar.

Discussion

The first cytological investigation of cell division in *Oedogonium* was by STRASBURGER (19), working with *O. tumidulum* Kg. in 1880. He described the origin of the ring, the whole process of nuclear division, and the formation of the cell wall. In the nuclear division he described some phases, but did not include the behavior of the chromosomes, a feature which was studied more accurately in 1908 by WISSELINGH (23), who also counted the chromosome number in *O. cyathigerum*. KLEBAHN (8) investigated the nuclear division in *O. boscil*. These investigators, comparing the mitosis of *Oedogonium* with that of higher plants, like *Tradescantia* or *Lilium*, agreed in finding striking resemblances between them.

It is not necessary to repeat the same details here, since they can only be confirmed, but there may be some small differences in my material. The one thing which should be mentioned is that my material, *O. grande*, shows more details in the mitotic figures. In the first place, modern methods of technique show structures more accurately than the former ones; and also there are surely differences

in the different species. Treating the different species with the same methods produces quite different results: some species show distinct figures; others show them obscurely; and some do not show anything, probably on account of abundant cell contents.

WISSELINGH (23) counted nineteen chromosomes in *O. cyathigerum*; I found thirteen in *O. grande*. He considered it a matter of interest that there was an uneven number in the species which he studied, a feature which he supposed to be very rare in the plant kingdom. I also found the forms of chromosomes in a figure to be as various as he described them in his species.

In regard to the origin, structure, and growth of the ring, as well as the formation and behavior of the separating wall between the two daughter cells, there have been various theories discussed by almost all the workers who have investigated *Oedogonium*, especially PRINGSHEIM (16), STRASBURGER (19), KLEBAHN (8), WILLE (21), HIRN (6), and WISSELINGH (24). The history of these investigations is well described by WISSELINGH, who also worked out the features in detail. Nothing entirely new was found in *O. grande*, but the investigation confirms especially WISSELINGH's descriptions of the origin of the ring, its manner of stretching, and the formation and behavior of the separating wall between two daughter cells.

Spermatogenesis was studied chiefly by PRINGSHEIM (16), JURANYI (7), KLEBAHN (8), and HIRN (6), but the descriptions were mostly morphological, not cytological. KLEBAHN described the details of formation of the antheridial cell, however, and made some remarks on the mitosis, but not in detail. Some figures of *O. grande* resemble his illustration, but differ in having the formation of a ring, and also in not having both vertical and horizontal divisions in the antheridial cells of the same filament. According to his explanation, an antheridium is forming sperms in both ways, namely, by vertical and horizontal divisions; while in my material another antheridial cell is being produced. In *O. grande* each primary antheridial cell produced by the mother cell usually forms another secondary antheridial cell, the so-called sperm mother cell with the lateral wall developed by a ring. This antheridial cell forms two sperms by a vertical division without forming a ring. My material fortunately shows an almost complete series of the mitosis in both the formation of

antheridia and sperms; although in the latter the figures are not so distinct as in the former, owing to the narrowness of the cells.

The most striking features are exhibited by the mitotic figures in the division of the antheridial cell. In this case the ring is formed as usual, but the mitotic figures lie along the transverse axis, in spite of the fact that horizontal cell division is going to take place. The reason may be that the length of the cell is too short to let the figures lie longitudinally. But finally, when the ring stretches out, one of the nuclei migrates into the new space. It seems to be an interesting phenomenon from the point of view of adaptation. Even minute figures like these adapt themselves to the environment for some time in the process of division.

In the formation of sperms, although the same mitotic process is repeated, one thing is different from typical *Oedogonium* cell division: there is no formation of a ring. The reason seems to be that the cell itself has already been formed, consequently division takes place only in the cell contents.

PRINGSHEIM, HIRN, and others investigated the development of dwarf males, but they did not try to trace the whole process step by step. The dwarf male is so small that it is hard to study the mitosis in detail, but a morphological tracing can be made without difficulty. The dwarf males of *O. nebraskense* form in general two antheridial cells; the upper one is always older than the lower, and each produces two sperms without the formation of a ring.

It is of interest to compare this nannandrous type with the macrandrous type in their methods of spermatogenesis. In the nannandrous type, the stipe corresponding to the antheridial mother cell produces only antheridial cells, which in this case never form another secondary antheridial cell. In the macrandrous type, however, both the antheridial mother cell and antheridial cell are generally able to form another antheridial cell. The length of the antheridial cell of both types is quite different, being much longer in the nannandrous type; therefore this might be the best form to study for the origin and development of the cilia of sperms. There is more space and comparatively less cell contents.

In oogenesis the principal work has been done by PRINGSHEIM, KLEBAHN, and HIRN, but in most of the forms studied there was no

suffultory cell; consequently I have described oogenesis with a suffultory cell to compare with the other accounts. The behavior of the nucleus is interesting, because it does not migrate at all toward the upper end of the cell, but nevertheless shows how active it is as the center of oogenesis.

Fertilization was described by PRINGSHEIM, JURANYI, KLEBAHN, and HIRN. KLEBAHN furnished an excellent description from the cytological point of view, which is confirmed by my observations in *O. americanum*. I have added a brief sketch of the formation of the cell wall of the fertilized egg, using the same species, which is quite different from other cases of cell wall formation in *Oedogonium*.

Formation of the zoospore has also been worked out by various investigators, among them STRASBURGER (18), who studied its cytology. He mentioned repeatedly the change of position of the nucleus during the various stages of development of the zoospore. My material, probably owing to the different species, shows scarcely any such changes of the nuclear position, except a slight migration inward from the peripheral position, where a clear circle appears. The larger the clear circle becomes, however, the deeper the nucleus sinks. The nucleus always accompanies the clear circle, never moving apart from it; consequently no radial substance was found between the nucleus and the circle, as STRASBURGER observed; but numerous coarse and fine granules were found around the circle, as if they were going to form a blepharoplast, as DAVIS (3) suggested in his study of *Derbesia*.

Germination of the fertilized egg was searched for constantly, but was not found in material just brought in from the field, and could not be induced by any of the methods which have proved successful in forcing the germination of seeds. No doubt reduction of chromosomes takes place at the germination of the egg. No one has ever observed the nuclear conditions at the germination of the egg of *Oedogonium*, or of the nearly related *Bulbochaete*.

Summary

1. Although the somatic mitosis of *Oedogonium* has already been shown by STRASBURGER and WISSELINGH, *O. grande* shows the figures more distinctly than their illustrations, furnishing a complete

series of the mitotic process, especially the behavior of chromosomes which show a remarkable resemblance to those of higher plants. This is due partly to modern methods of technique and partly to the species studied.

2. The number of chromosomes of this species is thirteen, an uneven number, in this respect agreeing with the uneven number nineteen in *O. cyathigerum* counted by WISSELINGH.

3. In the spermatogenesis all important phases of the mitosis in the formation of both antheridia and sperms have been traced in *O. grande*. The most striking feature is found in the mitosis of the antheridial cell, in which the figure is oriented along the horizontal axis, while the cell division is to be transverse. But when the ring stretches, one of the daughter nuclei, which have been lying side by side, moves into the upper space and becomes separated by a cross wall. This seems to be an unusual condition in cell division and has never been described in *Oedogonium*.

4. The complete series of spermatogenesis of the dwarf male was found in *O. nebraskense*, which shows the formation of sperms very distinctly.

5. There are two types of oogenesis, one with a suffultory cell (*O. nebraskense*) and the other without (*O. americanum*). The behavior of the nucleus in oogenesis is different from that in the vegetative cell division, in staying in the same position or in migrating slightly toward the lower end of the cell, while the nucleus in the vegetative cell division migrates toward the upper end of the cell. Both show that they are the center of morphological and physiological activity.

6. As soon as fertilization takes place in *O. americanum* the spore coat develops from the fertilized egg. The spore coat consists of three layers: a thin and smooth outer one; a thick, half wavy and half smooth middle one; and a thin and smooth inner one.

7. In the formation of the zoospore, the behavior of the nucleus of the unknown species is a little different from that of *O. tumidulum* described by STRASBURGER, in not changing its position according to the various stages of the development of the zoospore. The nucleus of my material sinks just under the clear circle in which the cilia of the zoospore appear. The process of escape of the zoospore from the

cell takes only a few minutes. Of the factors which stimulate the formation of the zoospore, change of temperature seems to be most effective so far as the present experiments are concerned.

Professor CHARLES J. CHAMBERLAIN suggested that I undertake this work, and I wish to express to him my gratitude for his valuable suggestions and kindly encouragement. I am also indebted to Professor E. N. TRANSEAU, who examined the material and determined the species.

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EXPLANATION OF PLATES I-III

FIGS. 1-28 (except fig. 3), $\times 790$; figs. 3, 29, $\times 380$; figs. 31-43, $\times 800$; figs. 30, 44, 46, 47, 49, 52, $\times 390$; figs. 45, 48, 50, 51, 53, 54, $\times 350$; figs. 55-69, $\times 800$.

FIGS. 1-28 (except figs. 20, 21, 23), *O. grande*; figs. 20, 21, 23, 29, unknown species; figs. 30-54 (except figs. 50, 51), *O. nebraskense*; figs. 50, 51, 55-64, *O. americanum*; figs. 65-70, unidentified species.

FIG. 1.—Vegetative cell with reticulate chromatophore.

FIG. 2.—Resting nucleus.

FIG. 3.—Migration of nucleus toward upper end of cell.

FIG. 4.—Elongation of nucleus and appearance of young ring.

FIG. 5.—Prophase: irregular zigzag threads of chromatin and reticulum of uneven thickness.

FIGS. 6, 7.—Condensation of chromatin threads to even thickness.

FIG. 8.—Single slender and coiled spireme splitting longitudinally by small vacuoles.

FIG. 9.—Segmentation of chromosomes and appearance of spindle.

FIG. 10.—Metaphase: gathering of chromosomes at equator of spindle.

FIG. 11.—Various forms of spindle attachment.

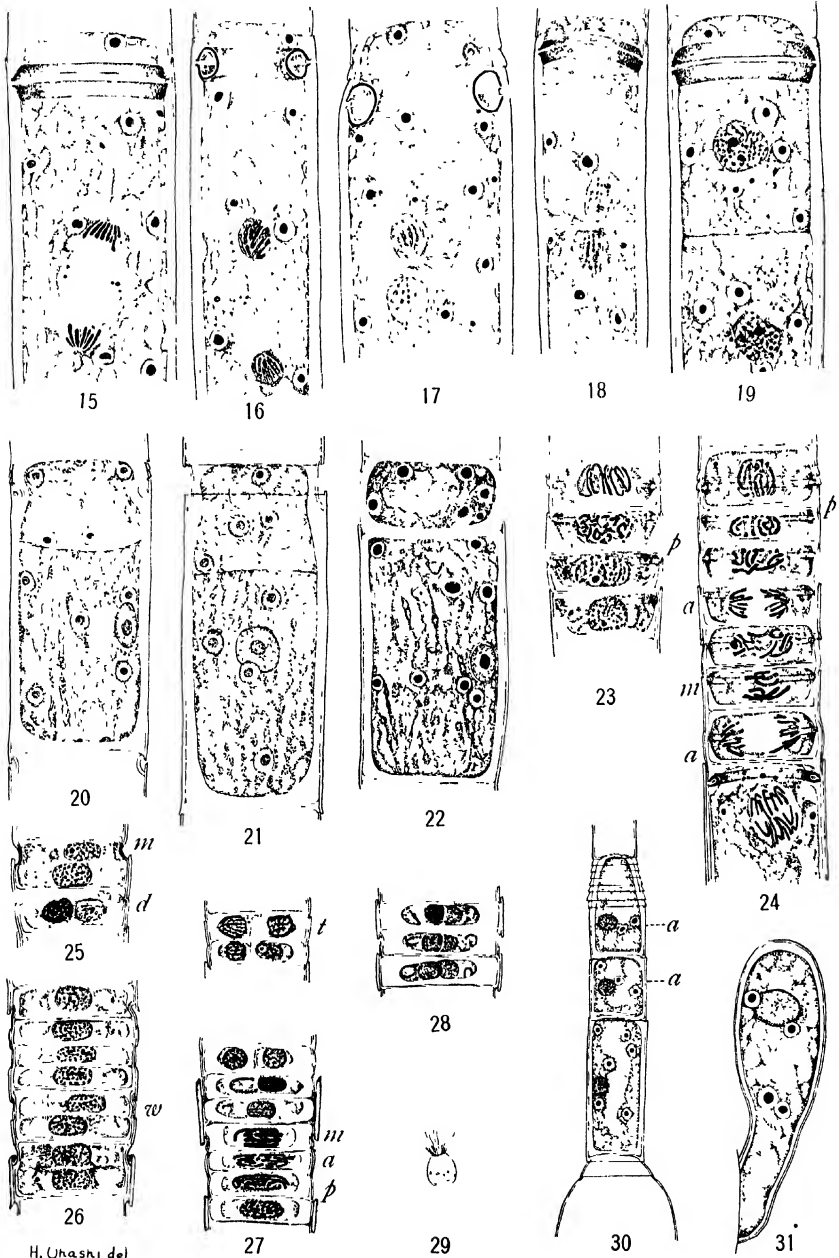
FIG. 12.—Anaphase: separation of double chromosomes.

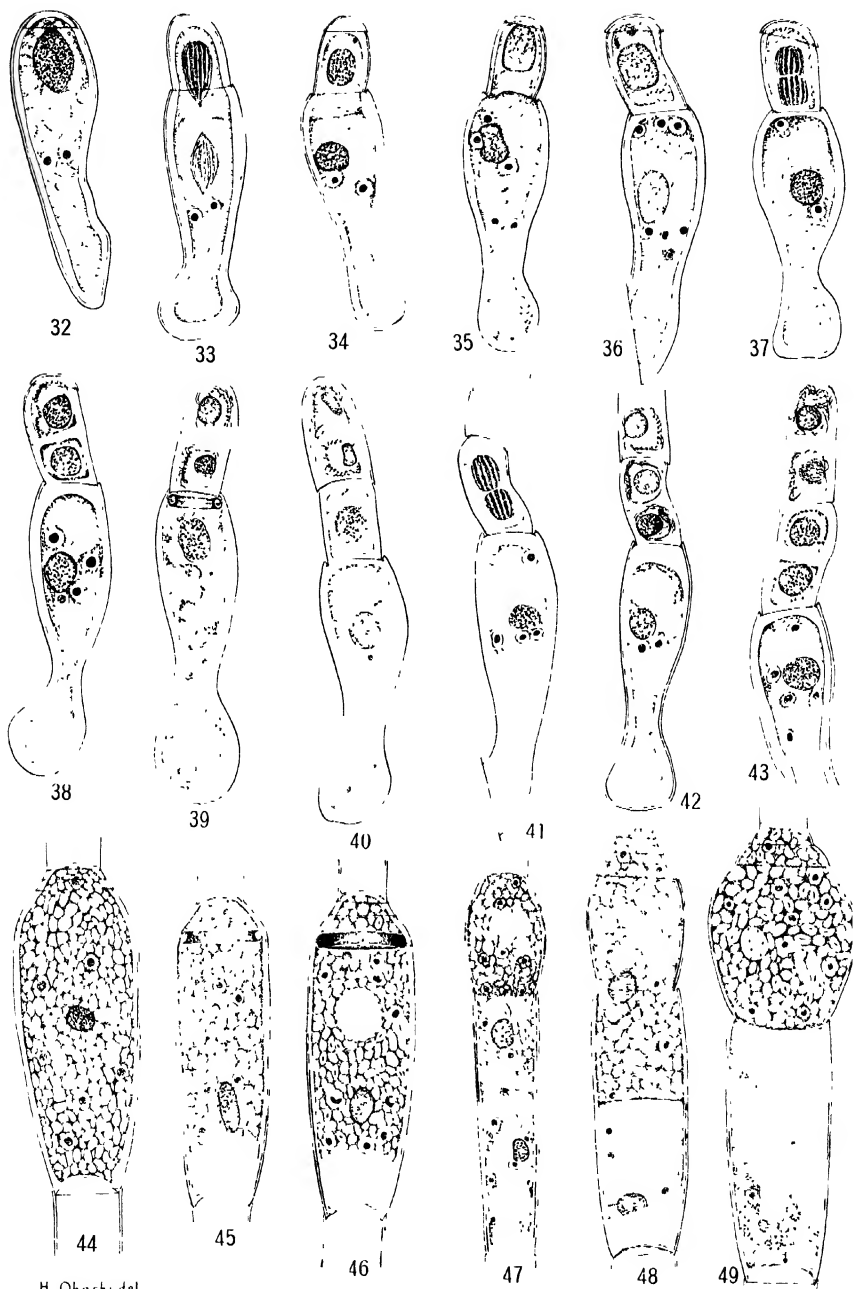
FIGS. 13, 14.—Migration of chromosomes to poles of spindle.



H. Ohashi del.

OHASHI on OEDOGONIUM





H. Ohashi del.

FIG. 15.—Contraction of chromosomes into masses.

FIG. 16.—Telophase: separation of chromosomes by vacuoles along axis for reconstruction of resting nucleus.

FIG. 17.—Approach of two daughter nuclei at equator of spindle.

FIG. 18.—Appearance of nucleolus in more or less uniform reticulum and formation of cross wall between two nuclei.

FIG. 19.—Separation of nuclei after formation of cross wall.

FIG. 20.—Beginning of stretching of ring under outer layer of cell wall, showing some mucilage at outside.

FIG. 21.—Broken outer layer of cell wall.

FIG. 22.—Straightening of ring and migration of cross wall upward to unite with inner layer of cell wall.

FIGS. 23, 24.—Mitotic figures lying horizontally in antheridial cells with formation of ring; showing various stages in prophase (*p*), metaphase (*m*), and anaphase (*a*).

FIG. 25.—*d*, Two daughter nuclei lying side by side in antheridial cell; *m*, migration of one nucleus upward after stretching of ring.

FIG. 26.—Formation of cross wall between two nuclei lying one above the other.

FIG. 27.—Mitosis in sperm mother cell showing prophase (*p*), metaphase (*m*), anaphase (*a*), and telophase (*t*).

FIG. 28.—Formation of sperms.

FIG. 29.—Swimming sperm.

FIG. 30.—Androsporangia (*a*) of *O. nebraskense*.

FIGS. 31-36.—Dwarf males of *O. nebraskense* in various stages of formation of first antheridial cell.

FIGS. 37, 38.—Formation of sperms in first antheridial cell.

FIG. 39.—Formation of second ring in stipe, and ciliated sperms in first antheridial cell.

FIG. 40.—Formation of second antheridial cell.

FIG. 41.—Formation of sperm in second antheridial cell.

FIGS. 42, 43.—Formation of sperms.

FIG. 44.—Oogonium mother cell of *O. nebraskense* with nucleus at center.

FIG. 45.—Formation of ring at top of oogonium mother cell and migration of nucleus toward lower end of cell.

FIG. 46.—Ring at full growth in mother cell.

FIGS. 47-49.—Various stages of oogenesis with suffultory cell.

SPECIALIZATION IN SECONDARY XYLEM OF DICOTYLEDONS

II. EVOLUTION OF END WALL OF VESSEL SEGMENT

FREDERICK H. FROST¹

(WITH SIXTEEN FIGURES)

Introduction

The origin of the vessel was discussed in the initial paper of this series (4). It was found that the vessel segment of the arboreal dicotyledons developed phylogenetically from a typical scalariform tracheid by the loss of pit membranes at the ends of the tracheid. The specialization also resulted in the formation of clearly defined end walls. The present paper continues the discussion from this point, and traces the further specialization of the primitive scalariform perforation. The first paper includes an explanation of the methods which were used in this and the following studies.

BOODLE and WORSDELL (2) described transitions from scalariform to simple perforations which they had observed in the secondary xylem of several dicotyledons. Although they did not state that the sequence reflects the phylogenetic origin of the porous vessel segment, this may be inferred as their viewpoint. JEFFREY (5) described an identical transition from *Vaccinium*. He concluded, from a consideration of the sequence, that the simple perforation originates from the scalariform perforation by the gradual loss of the bars of the latter. Later THOMPSON (6), studying *Vaccinium*, corroborated the interpretation of JEFFREY, and agreed that the sequence is palingenetic and that it illustrates the origin of the porous vessel segment.

BROWN (3) described similar transitions which he had observed in positional sequence from the protoxylem to the metaxylem. BAILEY and TUPPER (1) showed conclusively that the scalariform perforation is primitive and that the simple perforation is specialized. This proved, for the first time, that the sequence is truly palingenetic

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and that the direction of the specialization is from the scalariform to the simple condition.

Despite contradictory opinions as to the origin of the vessel, there is uniformity of opinion concerning the further specialization of the end wall. In the first place, the wide occurrence and uniformity of the sequence in many different plants indicate its palingenetic character, and, in the second place, there is only one rational manner in which to interpret the direction of the sequence. The present paper presents additional evidence in favor of this well established view, and outlines the details of this line of specialization.

Discussion

Dicotyledonous woods may be classified into four major groups on the basis of the type of vessel perforation. These are: (1) woods with vessels which have exclusively scalariform perforations; (2) woods with both scalariform and simple perforations in different vessel segments; (3) woods with oblique simple perforations and with vestiges of the scalariform condition in the smaller vessel segments; and (4) woods with vessels which have transverse porous division walls. The scalariform-porous woods may be dominantly scalariform, as is typical of *Myrica inodora*, or dominantly porous, a condition found in *Sambucus simpsonii* and many other species.

To obtain the evolutionary sequence of these four types, it is necessary to correlate them with a characteristic whose evolutionary history has been established. The writer showed, in the first paper of this series (4), that specialization resulted in a decided decrease in the length of the vessel segment. Using length of vessel segment as a base, the results given in table I were computed from data taken from BAILEY and TUPPER (1).

Table I shows that with increasing specialization of the vessel segment, the perforations of the end walls change from the scalariform to the simple type. It may be concluded, therefore, that the scalariform perforation is primitive and the porous perforation specialized, and that the phylogenetic order of development is from scalariform, to scalariform-porous, to oblique-porous with vestiges of the scalariform condition, to transverse porous. Scalariform-porous species with dominant scalariform perforations are, in regard

to this particular characteristic, more primitive than those with dominant porous perforations.

If the assumption is made that the vessel segment is a modification of the tracheid, it follows, by the method of association, that primitive vessel segments will possess the general features of tracheids. It also follows, by the method of correlation, that characteristics which correlate with these tracheid-like features will themselves be primitive in the organization of the vessel segment. The

TABLE I
AVERAGE LENGTH OF VESSEL SEGMENTS

TYPE OF PERFORATION	NO. OF SPECIES	LENGTH (MM)
I. Entirely scalariform	52	1 09
II. Scalariform porous	19	0 81
III. Oblique porous	34	0 69
IV. Transverse porous	169	0 41

TABLE II
CORRELATIONS OF PRIMITIVE CHARACTERISTICS WITH
TYPE OF PERFORATIONS

TYPE OF PERFORATION	NO. OF SPECIES	DIAMETER (MM)	PERCENTAGE		
			Outline angular	Thin walls	Evenly thickened walls
I. Scalariform . . .	49	0 067	100	100	97
II. Porous(transverse)	40	0 120	15	25	22

general features of tracheids are, in part, small cross-sectional diameter, angularity of outline, thin walls, and evenly thickened wall. The measurements summarized in table II were made as a check on the length-perforation correlation.

In every case the scalariform condition correlates with the primitive characteristic while the specialized or porous condition of the end wall shows little correlation. In addition to this evidence, many species show the transition from the scalariform to the simple perforation when a traverse is made from the protoxylem to the secondary xylem. This transition is also found in the secondary xylem, and is illustrated in fig. 1 from *Myrica inodora*.

There are five major factors which should be considered in a detailed investigation of this transition: (1) the nature of the border of the scalariform apertures and of the porous openings; (2) the number of the bars; (3) the width of the scalariform openings; (4) the inclination of the end walls; and (5) the relationship between the pitting of the end and side walls.

1. SCALARIFORM APERTURES. These are of four types: with a complete border, with a border complete to the middle of the orifice, with a border only at the ends of the orifice, and non-bordered apertures. There are, from this point of view, only two types of porous

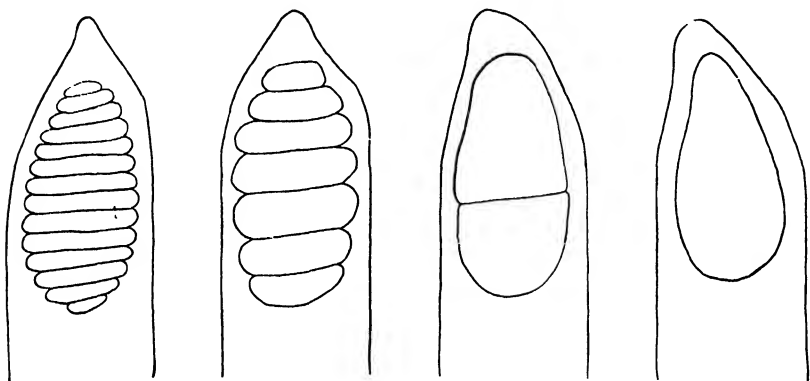


FIG. 1. *Myrica inodora*, four stages showing origin of simple perforation

perforations which are significant. The openings may be completely to partially bordered or non-bordered.

The pits of the scalariform tracheid type, which gave rise to the primitive vessel segment of the dicotyledons, are invariably bordered. The loss of the membrane from a pit of this nature would result in an orifice with a complete border, and such being the case, one would expect that the most primitive type of scalariform opening would be completely bordered, and that the loss of these borders would be evidence of specialization. Table III shows the correlation between the nature of the border of the orifice and the length of the vessel segment.

The vessel segments of group I, as shown by their great average length, are considerably more primitive than the vessel segments of

group II. It follows that the completely bordered type is more primitive than the partially bordered or non-bordered type. Table IV illustrates how well the specialization of the length of the vessel segment, and thereby all characteristics associated with length, correlates with the specialization of the border of the openings of the scalariform perforation. In the primitive condition of the perforation the border completely surrounds the openings; in the second stage the border is complete to the middle of the apertures only; in the third stage the border has disappeared from the sides and remains

TABLE III
WOODS WITH SCALARIFORM PERFORATIONS

TYPE OF ORIFICE	NO. OF SPECIES	VESSEL SEGMENT LENGTH (MM)
I. Completely bordered	16	1.34
II. Incompletely to non-bordered	35	0.98

at the ends of the openings only; and in the final transformation the border is entirely lost.

It would be possible to increase the number of cases used in forming these averages by increasing the number of species. This would involve taking more than one species from each genus, and would give an apparent, not a real stability, to the differences between the means. In all cases the actual differences between the means are more than three times their probable errors.

A considerable number of species exist with dominantly bordered perforations in the vessels of the secondary xylem, but very few in which all the perforations are bordered. This is because the completely bordered scalariform opening is the most primitive, and the wood of existing dicotyledons, with the exception of that of *Tetracentron*, etc., is considerably specialized. *Dillenia philippinensis*, whose vessel segments average 1.6 mm., is a good example of a wood in which the orifices are dominantly bordered.

Species with perforations which have borders to the middle of the orifice or only at the ends of the openings do not exist as a pure type, since the completely bordered condition is present as a vestigial characteristic or because the next type in the sequence is being in-

troduced. The primitive type is lost by slow stages and the advanced type enters in the same way. This lack of rigid lines of distinction between specific characters is responsible for the preservation of sequences which reflect the evolutionary history of certain characteristics. In *Hamamelis virginiana*, whose vessel segments average 0.8 mm. in length, the entire sequence from the fully bordered to the non-bordered condition may be constructed from individual variability (figs. 2, 3).

The situation at the end of the sequence is somewhat different since no new type appears, and since there has been sufficient time

TABLE IV
WOODS WITH SCALARIFORM AND SCALARIFORM-POROUS
PERFORATIONS

TYPE OF ORIFICE	NO. OF SPECIES	LENGTH OF VESSEL SEGMENT (MM.)
I. Completely bordered .	19	1 27
II. Bordered to middle	16	1 07
III. Bordered only at ends	38	0 82
IV. Non-bordered	32	0 57

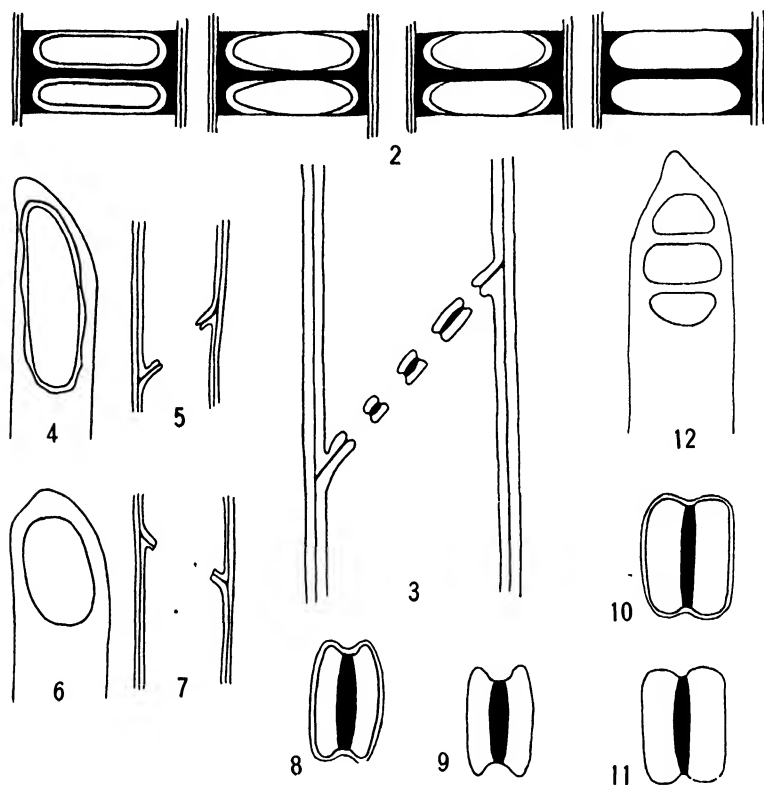
for the primitive types to disappear. *Alnus rubra*, whose average vessel segment length also is 0.8 mm., is an illustration of a type in which practically all of the perforations are non-bordered.

That the bordered porous opening is more primitive than the non-bordered (figs. 4, 5) is indicated by the fact that the former condition is common in scalariform-porous woods and rare in transverse porous woods, whereas the latter condition is rare in scalariform-porous woods and characteristic of transverse porous woods.

Since the length of the vessel segments of the dicotyledons is correlated with the type of perforation and also with the type of border of the apertures of the perforation, it follows that the type of border must be correlated with the type of perforation. This correlation is given in table V.

In addition to showing the correlation between the type of border and the type of perforation, table V shows several other points of interest. There are evidently two major lines of phylogenetic spe-

cialization, considering the relationships of the rates of specialization of the border of the aperture and the perforation as a whole: (a) the completely bordered to non-bordered transition may be concluded before the introduction of the porous perforation; and (b) the



FIGS. 2-12.—Fig. 2, *Hamamelis virginiana*, four stages in evolutionary loss of borders from scalariform apertures, fig. 3, *Hydrangea peruviana*, bordered scalariform perforation in section; figs. 4, 5, *Sambucus callicarpa*, bordered porous opening in surface and sectional view; figs. 6, 7, *Ehretia elliptica*, non-bordered porous opening in surface and sectional view; figs. 8, 9, *Viburnum tinoides*, completely bordered bar showing extra layer of wall substances, figs. 10, 11, *Alnus rubra*, slightly bordered bar showing same condition; fig. 12, *Paeonia moulan*, example of perforation with only a few bars.

porous perforation may be introduced before the border transition is fairly started.

(a) The first line of specialization is illustrated by the following diagram:

I	II	III	IV	V
Completely bordered	Bordered to middle	Bordered at ends	Non-bordered	Non-bordered
Scalariform	Scalariform	Scalariform	Scalariform	Porous

This is the only sequence which will account for scalariform woods with scalariform apertures bordered to the middle, bordered at the ends, and non-bordered. The number of scalariform species in groups I and II of table V is high relative to the number of scalariform-porous species, indicating that this part of the sequence is the

TABLE V*

TYPE OF BORDER	PERCENTAGE INITIALLY SCALARIFORM (PRIMITIVE)	PERCENTAGE SCALARIFORM-POROUS (INTERMEDIATE)
I. Complete (primitive)	89	11
II. Complete to middle (intermediate)	64	36
III. Bordered at ends (intermediate)	56	44
IV. Non-bordered (specialized)	50	50

* Based upon a study of 108 species

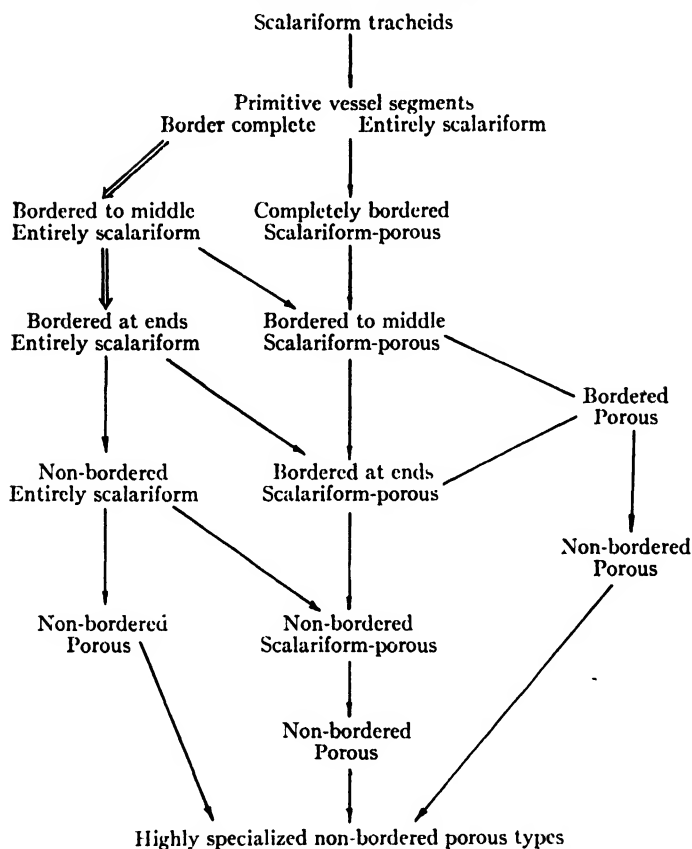
generally adopted pathway to the transverse porous perforation. The non-bordered scalariform perforation can give rise only to the non-bordered porous perforation, and therefore the bordered porous type could not have arisen from this sequence.

(b) The second line of specialization is more complicated, since offshoots of the first series may give rise to types in the second series. The main line is as follows:

I	II	III	IV	V
Completely bordered	Completely bordered	Bordered to middle	Bordered at ends	Non-bordered
Scalariform	Scalariform-porous	Scalariform-porous	Scalariform-porous	Scalariform-porous

This transition accounts for the scalariform-porous types which are dominantly bordered, and perhaps for the remaining types, although the others may be modifications of the major line of specialization.

The following diagram illustrates all the possibilities.



It will be noted that the bordered porous condition, which is not uncommon, can arise only from stages III and IV in the scalariform-porous transition. It is also probable that the step from the scalariform completely bordered type to the scalariform-porous completely bordered type is of rare occurrence. The bordered to middle scalariform-porous stage generally arises from the bordered to middle scalariform stage. This is indicated by the fact that the proportion of types I and II is higher in the scalariform series than in the scalariform porous series. Fundamentally, of course, the two major lines just given are the same, since they are differentiated only on the basis of the time of introduction of the porous perforation.

The membranes of a scalariform perforation, in the normal course of events, are lost after the cell has completed its ontogeny. The writer, however, has noticed both bordered and simple bars which are completely encircled by an additional layer or wall, which must have been deposited after the absorption of the membranes. These curious bars occur at random in all types of scalariform perforations and are of no evolutionary significance. Two varieties are illustrated in figs. 8-11.

2. NUMBER OF BARS. -- The bars composing a scalariform perforation may be many, intermediate, or few in number. If it is true that the scalariform tracheid gave rise to the vessel segment the

TABLE VI

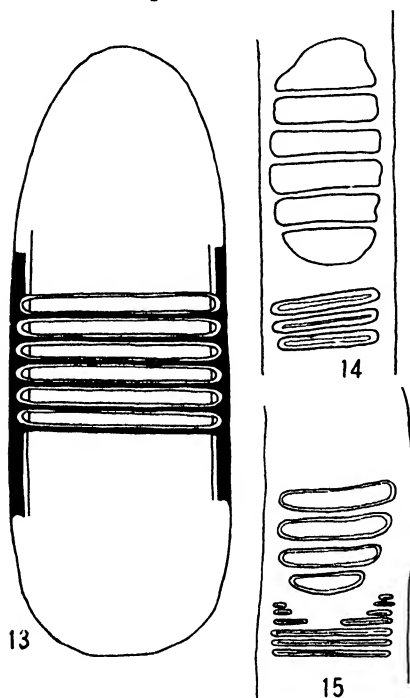
TYPE OF BORDER	SPECIES			PERCENTAGE OF MANY + INTERMEDIATE WITH FEW-BARRED TYPE
	Many	Intermediate	Few	
I. Completely bordered	8	6	4	77
II. Bordered to middle	7	4	5	69
III. Bordered at ends	6	19	13	65
IV. Non-bordered	0	14	18	43

many-barred type should be primitive, since the apertures of scalariform pits of tracheids are narrow and since the plane of contact between tracheids, in approximate vertical series, is long. The loss of pit membranes from a common wall of this nature would result in a many-barred perforation.

Defining roughly the many-barred type as over fifteen, the intermediate type as five to fifteen, and the few-barred type as five or less, table VI shows their correlation with the bordered to non-bordered sequence.

It is seen from table VI that the number of bars decreases with increasing specialization of the perforation, indicating that the many-barred type is primitive and the few-barred type is specialized. It is also pertinent to note that vestigial scalariform perforations are always characterized by a small number of bars. *Gordonia lasianthus* is an illustration of the many-barred condition, and *Paeonia moutan* is an excellent example of extreme reduction in bar number (fig. 12).

After the number of bars has been reduced to a very few, further specialization generally results in a loss of the remaining bars *in toto*, since intergrading forms, from the few-barred to the porous type, are rather unusual. This complete dropping out of the membrane of the perforation occasionally occurs at an earlier stage in the



FIGS. 13-15 —Fig 13, *Urandra luzoniensis*, showing occurrence of perforation which is scalariform on one side of membrane and porous on other; fig 14, *Talauma ovalis*, comparison of width of scalariform apertures with width of apertures of scalariform lateral pits; fig 15, *Bruguiera parviflora*, same as fig. 14.

area of the end wall. If the number of bars remained constant during this process they would gradually be crowded together and the apertures reduced in size. This would therefore result in a positive correlation between the resistance against the passage of a fluid and the phylogenetic development of the vessel. On the other hand, if the number of bars decreases faster than is necessary to maintain

the evolutionary development of the scalariform perforation. *Urandra luzoniensis* is an example of this variation. In this species the normal scalariform perforations are primitive and have numerous bars. Porous perforations are also present but the normal intermediate types are lacking. That the porous condition arises from the sudden loss of bars is indicated by the fact that perforations are common which are scalariform with many bars on one side of the membrane and porous on the other side of the membrane (fig. 13).

With increasing specialization of the vessel segment, as will be shown later, the end wall changes from a highly inclined to a transverse position, resulting in a considerable decrease in the length and

a constant width of aperture, the resistance to flow will decrease with the specialization of the vessel segment. It becomes evident, then, since the apertures do not decrease in size, that this line of specialization is advantageous to the ready flow of a liquid through the vessel. Whether this is advantageous to the plant as a whole is of course a different question, but one which might yield interesting results if tested experimentally.

Fig. 1, drawn from *Myrica inodora*, illustrates how the reduction in the number of bars is reflected in irregular variability in space in the secondary xylem of this species. (Note also figs. 6-10 of the first paper of this series.)

3. WIDTH OF SCALARIFORM OPENINGS.—In both the transitions referred to the width between the bars increases with specialization of the vessel segment. This certainly results in a decrease in resistance to flow and is accomplished by two factors which work in conjunction. The first cause which increases the width of the apertures is the loss of borders. It is not surprising that the borders disappear, since there seems to be no reason, other than the fact that the openings originated from scalariform pits, for their existence. When present, the borders partially fill the openings and increase the resistance to the passage of a fluid. The second and most important cause, which enlarges the openings of the scalariform perforation, is an actual reduction in the number of bars at a more rapid rate than that needed to maintain a constant size of aperture. *Talauma ovalis* and *Bruguiera parviflora* are excellent examples of the widening process. In *Talauma* the vessel segments are short and the borders often absent. The scalariform pitting of the side walls, however, has remained practically unchanged from the primitive condition. A comparison of the apertures of the side and end wall indicates the amount of phylogenetic widening of the openings of the scalariform perforation (fig. 14). In some instances the scalariform openings are more than ten times as wide as the apertures of the lateral pits. *Bruguiera parviflora* is a more primitive species with longer vessel segments. In this species the completely bordered condition is common, and in such cases the widening of the openings is due solely to a reduction in the number of bars (fig. 15).

It must be remembered that the actual width of the openings of

a scalariform perforation is correlated, to some extent, with the size of the vessel segment. In making comparisons of this characteristic, therefore, the size of the vessel segment must be taken into account. It is also true that the openings may increase only slightly during specialization of the vessel segment, and for this reason the wide opening is a much better indication of specialization than the narrow opening is of primitiveness.

4. INCLINATION OF END WALLS.—There is a strong correlation between the type of perforation and the inclination of the end wall. The primitive scalariform perforation is generally oblique, and the specialized porous perforation nearly always transverse. Table VII indicates the trend of this development.

TABLE VII
BASED ON 104 SCALARIFORM AND SCALARIFORM-
POROUS WOODS

TYPE OF PERFORATION	PERCENTAGE SPECIES WITH HIGHLY IN- CLINED END WALLS
I. Border complete	73
II. Partially bordered	41
III. Non-bordered	13

Table I shows that the porous species with oblique end walls are more primitive than the porous species with transverse end walls. The porous-oblique perforations are caused by a lag in the rate of change of the position of the end wall in relation to the time of introduction of the porous perforation. That is, if the change from scalariform to porous perforation occurs before the end wall has reached the transverse condition, an oblique porous perforation will result. That this is the normal case is shown by the great preponderance of oblique porous end walls in scalariform-porous species.

If the assumption is made² that the vessel segment is frequently subjected to collapsing pressures, due to tension of the liquid column, specialization of the end wall is productive of greater rigidity. A brace parallel to the direction of force, such as the transverse end wall, is better adapted to resist crushing forces than an

² In accordance with H. H. DIXON's theory of the ascent of sap.

oblique brace, and in addition the shortening of the vessel segment produces a greater number of braces per unit of length. It is not impossible to suppose that the huge vessels could not have evolved, in regions of high transpiration, if the vessel segment had not shortened in length and the perforation become transverse.

5. PITTING OF END AND SIDE WALLS.—BROWN (3) contends that the rate of specialization of the end wall would be slower than the

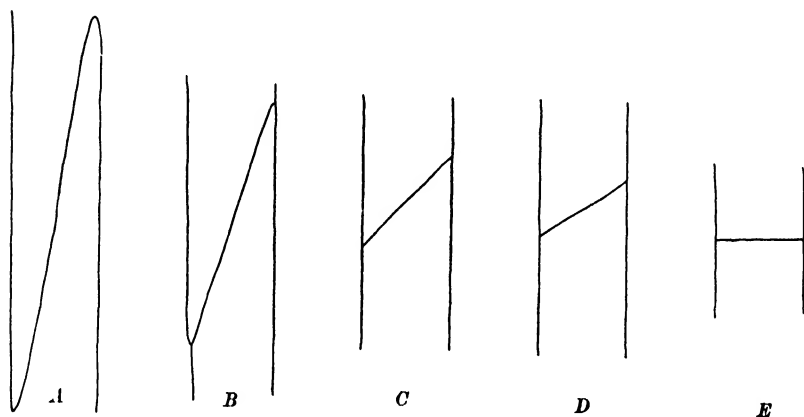


FIG. 16.—Evolution of inclination of end wall: (a) *Saurauja oldhami*, (b) *Panax edgerlegi*, (c) *Gilbertia affinis*, (d) *Diospyros virginiana*, (e) *Hicoria glabra*.

rate of specialization of the side wall since the end wall always separates tracheary elements, whereas the side wall comes into contact with a variety of cell types. In 51 entirely scalariform species, 29 have both scalariform side and end walls, while 22 show a distinct change in pitting from the end to the side wall. In 45 scalariform-porous species only four have scalariform lateral walls. These data would indicate that BROWN is correct. While these rates are undoubtedly of different magnitude, it must be remembered that slightly different rates will produce different results only after acting over a period of time, and this explains why the majority of scalariform woods have vessel segments with scalariform lateral pitting.

The occurrence and development of the reticulate, multiperforate, etc., types of perforations are not discussed in this paper, since the matter has been covered in detail by THOMPSON (7).

Summary

1. The scalariform perforation is primitive and the porous perforation is specialized. The phylogenetic order of development is: scalariform, scalariform-porous, oblique porous with vestiges of the scalariform condition, and transverse porous.
2. The primitive fully bordered aperture of a scalariform perforation gradually loses its border as the perforation becomes specialized.
3. The primitive scalariform perforation has many bars, which are generally lost in slow stages in correlation with the evolutionary development of the perforation.
4. Specialization of the scalariform perforation frequently results in a widening of the apertures of the perforation.
5. The inclination of the end wall changes from the highly inclined position to the transverse position as the scalariform perforation develops into the porous perforation.
6. The lateral pitting specializes more rapidly than the specialization of the perforation.

The writer wishes to take this opportunity to thank Professor I. W. BAILEY for his continued advice and criticism.

CUMBERLAND MILLS
MAINE

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MICROSPOROGENESIS IN THE CUCURBITACEAE

SARA F. PASSMORE

(WITH FORTY FIGURES)

Comparatively little work has been done on the development of the pollen mother cell of members of the Cucurbitaceae. STRASBURGER (10), in work on the determination of sex, refers to conditions in *Bryonia alba*; BOENICKE (2) and LUNDEGARDH (8), in their work on the heterotypic cell division, have studied members of this group, the former, *Bryonia dioica*, and the latter, *Cucurbita pepo*. *B. alba* and *B. dioica* have been studied by MEURMAN (9) in his work on sex chromosomes. None of these workers has given a detailed account of microsporogenesis. KIRKWOOD (6), however, has fully described the process in *Micrampelis lobata*. CASTETTER (3) has described it in *Cucurbita maxima*, and HEILMICH (5) in *Cucumis sativus*. KOZHUCHOW (7), although his work has been chiefly on the vegetative cells, studied the chromosomes of *Citrullus vulgaris*, *Cucumis melo*, *C. sativus*, *Cucurbita maxima*, *C. moschata*, and *C. pepo*. The reported chromosome numbers in the Cucurbitaceae are listed in table I.

Materials and methods

The following commercial varieties, arranged according to BAILEY'S (1) classification, were obtained from Dreer and Company, Seedsmen:

<i>Cucurbita pepo</i>	Jersey White Bush squash
<i>Cucurbita pepo</i>	English vegetable marrow
<i>Cucurbita maxima</i>	Warted Hubbard squash
<i>Citrullus vulgaris</i>	Kleckley Sweets watermelon
<i>Luffa cylindrica</i>	Luffa gourd
<i>Cucumis melo</i>	Rockyford cantaloupe
<i>Cucumis sativus</i>	White Spire cucumber

The plants were grown in the field, and fixations of the staminate buds were made at different hours of the day and night. Fixations made between 7:00 and 8:00 A.M. yielded more reduction division figures than those made at other hours, although cells in hetero-

typic division were found at other times also. Buds 1-8 mm. in length were fixed, the 5 mm. ones proving most fruitful in the plants of *Cucurbita*. In all other genera studied, buds about 2 mm. long contained cells in reduction division stages. The perianths were removed in the larger buds. The smaller ones were cut longitudinally with a sharp knife before being placed in the fixative.

TABLE I
REPORTED* CHROMOSOME NUMBERS IN THE CUCURBITACEAE

SPECIES	1n CHROMOSOMES	2n CHROMOSOMES
<i>Bryonia alba</i>	10 (Boenicke, Meurman)	
<i>Bryonia dioica</i>	10 (Strasburger, Meurman)	
<i>Citrullus vulgaris</i>	11 (Passmore)	22 (Kozhuchow, Passmore)
<i>Cucumis melo</i>	12 (Passmore)	24 (Kozhuchow)
<i>Cucumis sativus</i>	7 (Heimlich)	14 (Kozhuchow, Heimlich, Passmore)
<i>Cucurbita maxima</i>	20 (Castetter, Passmore)	40 (Castetter)
<i>Cucurbita maxima</i>		44-48 (Kozhuchow)
<i>Cucurbita moschata</i>		48 (Kozhuchow)
<i>Cucurbita pepo</i>		24 (Lundegardh)
<i>Cucurbita pepo</i> var. <i>pomiformis</i>		40 (Kozhuchow)
<i>Cucurbita pepo</i> var. <i>citrullina</i>		42 (Kozhuchow)
<i>Cucurbita pepo</i> (Jersey White Bush)	20 (Passmore)	
<i>Cucurbita pepo</i> (English vegetable marrow)	20 (Passmore)	
<i>Luffa cylindrica</i>	11 (Passmore)	
<i>Micrampelis lobata</i>	16 (Kirkwood)	

* See Literature Cited at end of paper for references.

Various modifications of Flemming's, Bouin's, and Benda's solutions were tried, and the following Flemming type solution seemed most valuable: chromic acid (10 per cent) 0.40 cc., acetic acid (10 per cent) 4.00 cc., osmic acid (2 per cent in 2 per cent chromic) 3.00 cc., water 16.60 cc., maltose 0.3 gm. The Benda type solution which gave exceptionally good results in a few cases, but on the whole did not seem as dependable for the material as the preceding, was: 1 per cent chromic acid 16 cc., 2 per cent osmic acid 4 cc., glacial acetic acid, 2 drops.

The material was imbedded in paraffin, and sections from 7 to 20 μ in thickness were cut and stained with Haidenhain's iron-alum

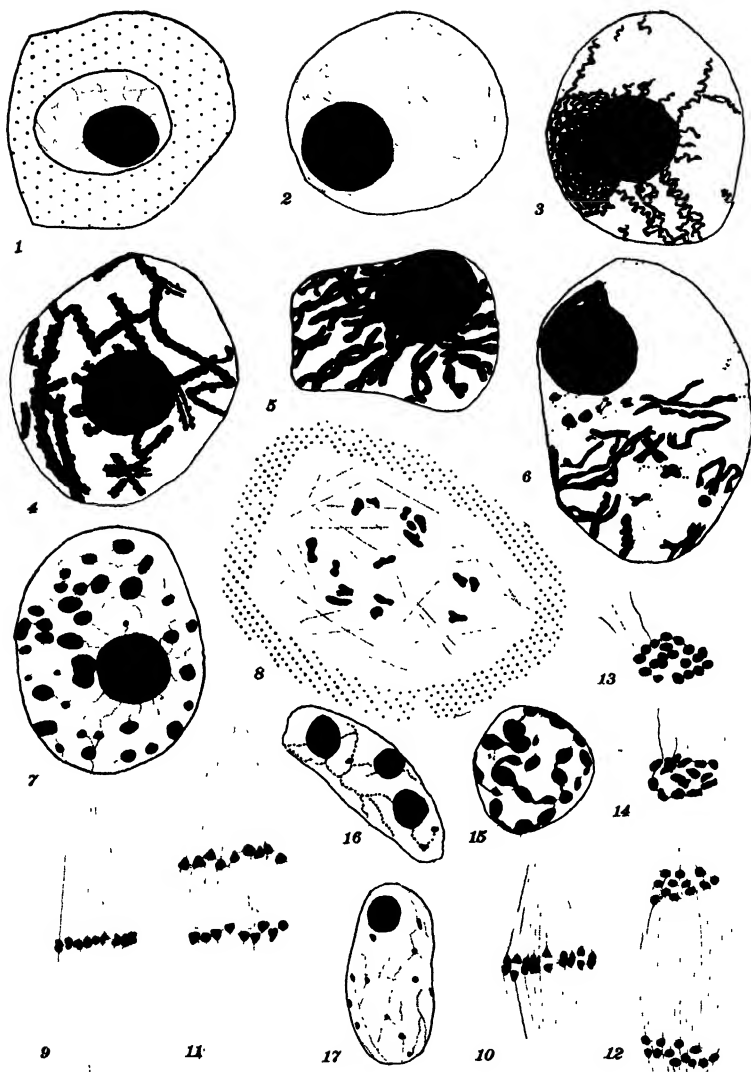
haematoxylin. Drawings were made with the help of the camera lucida.

This paper describes the development of the pollen mother cell of *Cucurbita pepo* (Jersey White Bush squash) from presynizesis to the quartet stage, and also shows the chief differences noted in microsporogenesis of the other varieties.

CUCURBITA PEPO

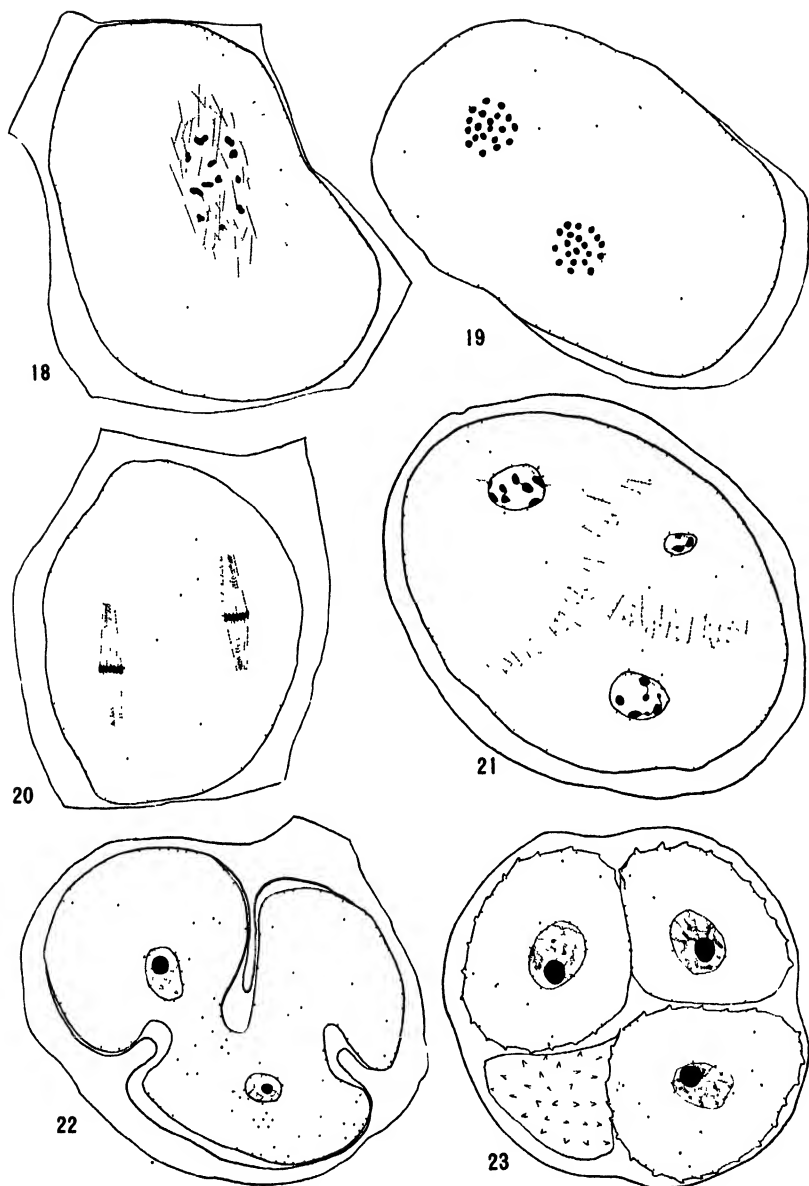
The cytoplasm in the primary sporogenous cells, which become the spore mother cells, is very dense. The nucleus contains a large deeply staining nucleolus, but the chromatin is rather meager and stains only faintly (fig. 1). Both the nucleus and the cell enlarge rapidly. The leptotene threads show some tendency to pair (fig. 2), but it is impossible to tell whether the apparent pairing which occurs here and there is significant or simply accidental. The spireme still stains rather faintly, but its staining property increases as it enters synizesis (fig. 3). When the spireme emerges from synizesis it has a beaded appearance and its doubleness shows plainly. It already seems to have divided transversely into segments of various lengths (fig. 4). The double segments twist themselves in ropelike fashion, and the beaded appearance becomes lost (fig. 5). At this stage the nucleolus is at its maximum size, but there seems to be a tendency for the nucleus as well as the cytoplasm to contract. The segments soon loosen and lie scattered through the nucleus (fig. 6), each apparently breaking into several parts (fig. 7). The nucleolus by this time is much reduced in size. It is at this stage of development of the nucleus that the dense perinuclear zone so conspicuous at metaphase first makes its appearance.

Fig. 24 shows the condition of the nucleus just before the membrane disappears. The pairs of chromosomes lie scattered through the nucleus, and among them are many darkly staining bodies. The nucleolus has disappeared at this time. When the nuclear membrane disappears, the spindle fibers, which seem to be partly of nuclear and partly of cytoplasmic origin, appear among the pairs of chromosomes (fig. 8). From this multipolar stage the spindle gradually becomes bipolar (fig. 18), and the pairs of chromosomes are arranged at the equator (fig. 9). A polar view at metaphase shows



FIGS. 1-17.¹—Fig. 1, young pollen mother cell; fig. 2, nucleus showing leptotene threads; fig. 3, synizesis; fig. 4, pachynema, segments showing doubleness; fig. 5, strepsinema, chromosomes twisted about one another; fig. 6, early diakinesis; fig. 7, late diakinesis; fig. 8, multipolar spindle with double chromosomes in meshes; fig. 9, first metaphase; fig. 10, end of metaphase, homologous chromosomes separating; fig. 11, anaphase showing attenuated form of chromosomes as they pass to poles; fig. 12, late anaphase; fig. 13, twenty chromosomes at pole of spindle; fig. 14, early telophase (chromosomes have lost rounded appearance); fig. 15, nuclear membrane formed at telophase, chromatin and nucleoli reorganizing; fig. 16, late telophase showing three nucleoli; fig. 17, nucleus at end of first division.

¹ All figures are of Jersey White Bush squash, $\times 1500$.



FIGS. 18-23.²—Fig. 18, spindle becoming bipolar, paired chromosomes arranging themselves at equator; fig. 19, polar view of second metaphase; fig. 20, second metaphase, spindles parallel; fig. 21, late second telophase; fig. 22, furrows dividing cell into quartets; fig. 23, microspores within sporocyte wall.

²All figures are of Jersey White Bush squash, except fig. 19, which is English vegetable marrow; $\times 750$.

twenty bivalent chromosomes (fig. 25). KOZHUCHOW (7) reports forty for the vegetative cells of *Cucurbita pepo* var. *pomiformis* and forty-two for var. *citrullina*. LUNDEGARDH (8) reports twenty-four for the vegetative cells, a number which TISCHLER (11) thinks doubtful.

The homologues separate and pass to the poles with no evidence of lagging (figs. 10-12). The spindle attachments give the chromosomes an attenuated appearance as they move apart. Soon after the chromosomes reach the poles (fig. 13) they lose their rounded appearance and become angular and elongated (fig. 14). The nuclear material assumes a linklike appearance (fig. 15) in the reorganization of chromatin and nucleoli, as the membrane forms at telophase. Several nucleoli are formed at first (fig. 16), but usually only one is present at the end of the first division (fig. 17). This stage is quickly followed by second metaphase. The two spindles may be parallel (fig. 20) or they may occupy various other planes. Fig. 21 shows the pollen mother cell in second telophase. Division into quarters takes place by furrowing. Quadripartition is simultaneous in most cases (fig. 22). Occasionally furrowing seems to be more rapid in one plane than in the other, so that the cell is divided into two parts before complete quadripartition takes place. The microspores develop their characteristic markings while still inside the sporocyte walls (fig. 23).

During development of the pollen mother cell considerable growth takes place in the protoplast. The diameter of the protoplast in four stages of development was obtained by averaging the maximum and minimum diameters of five cells. The result was as follows:

Archeporium	Synizesis	First metaphase	Second telophase
24 μ	48 μ	58 μ	65 μ

This method of obtaining the size of the cells is not strictly accurate, but at least gives an idea of the great change in size that takes place.

The development of the pollen mother cell in the English vegetable marrow is identical with that of the other variety of *Cucurbita pepo*, so far as the writer could ascertain. The chromosome number is likewise twenty (fig. 19).

CUCURBITA MAXIMA

This species seems also to be identical in its development of the pollen mother cell with that of *Cucurbita pepo* just described. CASTETTER (3) described microsporogenesis in this species, from the development of the spindle to the quartet stage. The writer's material seems to be in agreement with his observations, except that he reports cell plates which were not observed in this material. The haploid chromosome number is twenty (fig. 26). KOZHUCHOW, working on the root tips of *C. maxima*, reports 44-48 chromosomes as the diploid number. One of the difficulties in work of this kind, however, lies in establishing the fact that varieties and species that are given the same name are actually identical.

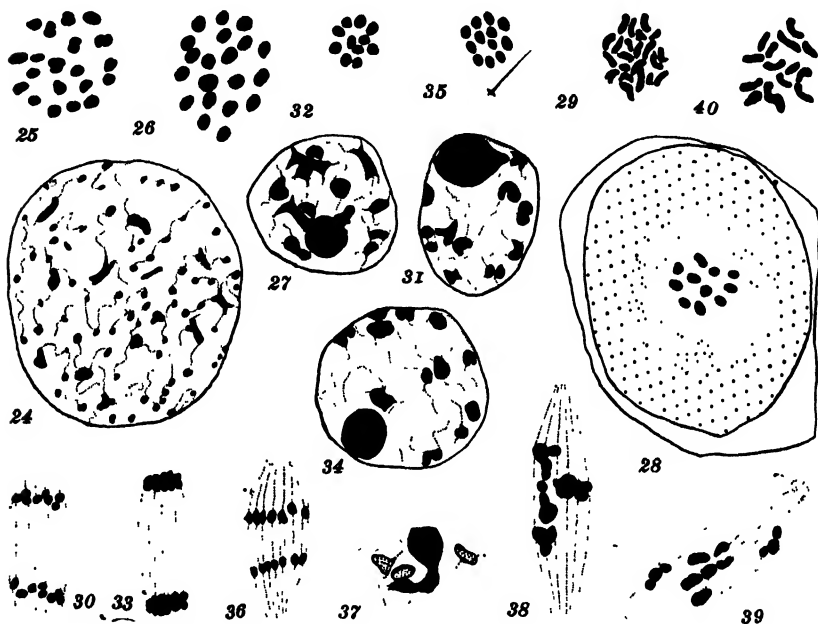
CITRULLUS VULGARIS

In all stages of development, the pollen mother cells of this and of the plants that follow are much smaller than those of *Cucurbita*. A cell at metaphase in this genus is only about one-fourth the size of the cell in the corresponding stage in *Cucurbita*. So far as the writer could ascertain, development up to diakinesis proceeds as in *Cucurbita pepo*. Here the paired chromosomes (fig. 27) can be seen lying about the nucleus, but there are no extra darkly staining granules among them, as in *C. pepo*. The nucleolus remains intact until the membrane disappears. A polar view at metaphase shows eleven bivalent chromosomes (fig. 28). Root tips show twenty-two chromosomes (fig. 29), the same number reported by KOZHUCHOW. At anaphase the chromosomes separate promptly with no evidence of lagging (fig. 30).

LUFFA CYLINDRICA

The chief difference noted in the development here is that at diakinesis the bivalent chromosomes scattered through the nucleus (fig. 31) lack the darkly staining bodies so evident in *Cucurbita pepo*. The nucleolus shows a tendency to bud, but is intact until the membrane disappears. It is sometimes found among the spindles at the multipolar stage, at which time, however, it is much reduced in size. There are eleven bivalent chromosomes (fig. 32), the number apparently not having been reported before. At anaphase the homologues separate and pass promptly to the poles with no evi-

dence of lagging (fig. 33). The appearance of the chromosomes in *Luffa* is similar to that in *Citrullus*. It is interesting to note in this connection that the seeds of *Luffa* are almost the same as those of *Citrullus* in size and markings. The pollen grains of the two are also practically identical.



FIGS. 24-39.—Fig. 24, *Cucurbita pepo*: late diakinesis showing paired chromosomes among other darkly staining bodies; fig. 25, same: polar view of first metaphase showing twenty bivalent chromosomes; fig. 26, *Cucurbita maxima*: polar view of second metaphase showing twenty chromosomes; fig. 27, *Citrullus vulgaris*: late diakinesis showing eleven paired chromosomes; fig. 28, same: polar view of pollen mother cell at first metaphase showing perinuclear zone and eleven chromosomes; fig. 29, same: polar view of root tip cell showing twenty-two chromosomes; fig. 30, same: first anaphase; fig. 31, *Luffa cylindrica*: diakinesis showing ten of the eleven pairs of chromosomes, nucleolus budding; fig. 32, same: polar view of first metaphase showing eleven chromosomes; fig. 33, same: late anaphase; fig. 34, *Cucumis melo*: diakinesis, twelve pairs of chromosomes; fig. 35, same: polar view of first metaphase showing twelve chromosomes; fig. 36, same: first anaphase; fig. 37, *Cucumis sativus*: multipolar stage showing paired chromosomes among spindle fibers, nucleolus budding; fig. 38, same: chromosomes arranged irregularly on spindle; fig. 39, same: spindle showing two trivalent chromosomes passing to poles; fig. 40, same: polar view at metaphase in root tip cell showing fourteen chromosomes.

¹ All figures are $\times 1500$, except fig. 26, which is $\times 2130$.

CUCUMIS MELO

The pollen mother cell of *Cucumis melo* at diakinesis (fig. 34) has much the appearance of that of *Citrullus* and *Luffa*. Darkly staining granules are not present among the pairs of chromosomes. The nucleolus disappears with the disappearance of the membrane in all cases noted. Polar views at metaphase show twelve bivalent chromosomes (fig. 35). KOZHUCHOW reports twenty-four for the root tip cells. There is no evidence of lagging as the chromosomes pass to the poles at anaphase (fig. 36).

CUCUMIS SATIVUS.

Many of the pollen mother cells in the anthers of this species degenerate before diakinesis. About half of the contents produce pollen that looks normal, although none of it was tested for fertility. At diakinesis the nucleolus is very prominent and stains deeply. The chromosomes stain faintly, but can be seen as irregular masses lying about the nucleus. At the time the nuclear membrane disappears and the spindle fibers appear, the nucleolus loses its rounded appearance and sends out tubelike projections (fig. 37). The irregular projections of the nucleolus are often difficult to distinguish from chromosomes, since the latter by this time also stain deeply. HEIMLICH states that the nucleolus of *Cucumis sativus* "becomes much elongated or vermiform." He mentions the fact that the disappearance of the nucleolus is associated with the chromosomes at the multipolar stage. He also states that on account of the sizes and shapes of the elongations of the nucleolus, parts of it may be mistaken for chromosomes. As the spindle becomes bipolar the irregularly shaped masses of chromatin are distributed along it (fig. 38). There seems to be a tendency for some of the chromosomes to assume a trivalent formation (fig. 39), such as DARLINGTON (4) figures for certain hybrid species of *Prunus*. The chromosome count could not be ascertained definitely in the pollen mother cell of this material. HEIMLICH reports seven chromosomes; root tip counts show fourteen (fig. 40), the number given by both HEIMLICH and KOZHUCHOW for vegetative cells. Certain cells in the perilem have twenty-eight chromosomes. In many of the root tip cells late wall

formation occurs, so that two nuclei frequently appear in the same cell. The nucleolus in root tip cells disappears by budding in much the same manner as in the pollen mother cell.

Summary

1. Darkly staining granules are present among the bivalent chromosomes in late diakinesis in all members of *Cucurbita*, but are absent in *Citrullus*, *Luffa*, and *Cucumis*.

2. *Cucurbita pepo* and *C. maxima* each has twenty bivalent chromosomes.

3. *Citrullus vulgaris* has eleven bivalent chromosomes; *Luffa cylindrica*, eleven; *Cucumis melo*, twelve. *Cucumis sativus* has fourteen chromosomes in the diploid cells.

4. The homologous chromosomes pass to the poles at anaphase with no evidence of lagging in *Cucurbita pepo*, *C. maxima*, *Citrullus vulgaris*, *Luffa cylindrica*, and *Cucumis melo*.

5. Chromosomes are distributed irregularly on the spindle at anaphase in *Cucumis sativus*.

6. The nucleolus shows a tendency to bud in both *Luffa cylindrica* and *Cucumis sativus*, and is often present after the spindle fibers appear in these species.

I wish to express appreciation to Professor W. R. TAYLOR for his interest and assistance in this work.

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THE MICROFLORA OF LEACHED ALKALI SOIL

J. E. GREAVES AND J. DUDLEY GREAVES¹

Introduction

It is generally recognized that a soil to which a soluble salt has been added is not restored to its original condition merely by leaching. There have resulted far reaching physical, chemical, and biological changes in the properties of the soil, and in many cases it is with difficulty, if at all, that it can be restored approximately to its native condition. Moreover, it has been demonstrated that the microflora of a soil can be profoundly modified both qualitatively and quantitatively by the use of soluble salts, the extent depending upon the specific salt, its concentration, and the bacterial activity of the original soil. Some soluble salts in comparatively low concentration greatly retard or even check beneficial bacterial processes. Many soluble salts when in soils in low concentration greatly accelerate some beneficial bacterial activities. This may be due to their action upon the structure of the soil or the liberation of essential nutrients, and probably less often to a direct stimulating action of the salt upon the bacterial protoplasm. Hence the microflora of an alkali soil should be far different from that of a non-alkali soil.

Some soils, even after leaching, remain barren or produce only meager crops for a number of years. The non-productive period may be due to physical, chemical, or biological changes which have occurred within the soil owing to its alkali content or the changes incidental to the leaching process. It is not unreasonable to expect the microflora to be likewise greatly influenced, and it would require considerable time after the leaching of such a soil before a normal active microflora would become established. This non-productive period may be shortened by proper inoculation with productive soil.²

In order to gain more precise information on the microflora of leached alkali soil, during the past few years rather extensive studies

¹ Contribution from Department of Bacteriology and Chemistry, Utah Agricultural Experiment Station. Publication authorized by Director June 13, 1929.

² GREAVES, J. E., The influence of salts on bacterial activities of soil. *BOT. GAZ.* 73:161-180. 1922.

have been conducted upon the specific microorganisms and their activity in leached alkali soil. Numerous studies have been made on various alkali soil, both naturally occurring and synthetically prepared, but the results reported in this paper were obtained on three soils: (1) a synthetic alkali soil made non-productive by the addition of 0.66 per cent each of sodium chloride, sodium sulphate, and sodium carbonate; (2) a naturally occurring alkali soil rich in chlorides and containing comparatively small quantities of the other soluble salts; (3) a natural occurring alkali soil rich in sulphates and containing only comparatively small quantities of other soluble salts. All three soils contained sufficient soluble salts to render them barren to all vegetation except a few salt-tolerant plants.

Investigation

The soils were packed into 2-gallon jars provided with a $\frac{1}{2}$ -inch hole in the bottom for leaching. They were then permitted to stand

TABLE I
COLONIES DEVELOPING UPON SYNTHETIC AGAR FROM LEACHED ALKALI SOIL

SOIL	COLONIES DEVELOPING IN 7 DAYS		
	Just after leaching (thousands)	After crop of crimson clover (thousands)	After crop of barley (thousands)
Native	2573	3300	2250
Synthetic alkali	3042	10,250	2600
Synthetic alkali, leached	7200	9450	2650
Chloride	220	600	1800
Chloride, leached	885	3150	5450
Sulphate	1452	6000	3550
Sulphate, leached	1722	5050	3000

2 months with a moisture content of 20 per cent so that the soil would become packed and the various reactions tend toward equilibrium, after which they were leached for a period of 2 years, that is, until most of the salts which could be washed out by mere leaching were removed. They were planted after leaching to crimson clover and later to barley. Before each cropping the soils were sampled and analyzed as to numbers, ammonifying, nitrifying, and nitrogen-fixing powers. Finally the specific microflora which develop on synthetic Ashby media were studied in detail.

The addition of soluble salts to a calcareous loam and then the

leaching out of these salts greatly increased the number of micro-organisms obtained from this soil by the plate method. Apparently the soluble salt and the water had removed some substance from the soil which keeps in check its natural microflora,³ however, there is a direct stimulation of the bacteria by the remaining salts. This probably plays a major rôle in the change in numbers, but the results as a whole show the inadequacy of numbers as determined by the plate method for evaluating the productivity of soils. The ammonifying powers of the soil are more valuable for this purpose.

TABLE II

MILLIGRAMS OF AMMONIA PRODUCED IN 4 DAYS IN VARIOUS ALKALI SOILS
CONTAINING 1 PER CENT DRIED BLOOD

SOIL	MG. AMMONIA PRODUCED IN 4 DAYS		
	Directly after leaching	After crop crimson clover	After crop barley
Native	89	91	66
Synthetic alkali	19	10	34
Synthetic alkali, leached	64	67	73
Chloride	2	2	5
Chloride, leached	40	16	57
Sulphate	11	11	50
Sulphate, leached	24	29	66

The addition of 0.66 per cent each of sodium chloride, sodium sulphate, and sodium carbonate to this highly productive calcareous loam reduced its ammonifying powers three-fourths, and leaching the soil brought it back to 80 per cent normal with a tendency for the ammonifying powers to increase after cropping.

The chloride-rich soil produced very small quantities of ammonia but after leaching produced 41 per cent of that produced by the check productive soil. The sulphate-carrying soil was producing more ammonia than the chloride-carrying soil, with an average of 24 mg. of ammonia. After leaching it was producing only 49 per cent of normal. It is evident, therefore, that during the time these soils were under observation, three years, the ammonifying powers of leached alkali soils did not reach that occurring in normal productive soils. It was found that it could be brought to normal, however, by the addition of soil extracts, farm manures, and plant residues. The

³ GREAVES, J. E., Soil microbial stimulants. Proc. 1st International Congress Soil Science 1927. 3:222-228. 1928.

effect upon the nitrifying powers of the soils is even greater than upon the ammonifying powers.

At first the nitrifying power of the synthetic alkali soil was extremely low. Later, due to increased aeration, it became more active. The leaching of this soil not only restored its natural nitrifying powers but acted as a stimulant. It is evident that 0.66 per cent each of sodium chloride, sodium sulphate, and sodium carbonate is not sufficient to destroy the nitrifying bacteria, for when the salts are

TABLE III

MILLIGRAMS OF NITRIC NITROGEN PRODUCED IN 21 DAYS IN VARIOUS
ALKALI SOILS CONTAINING 1 PER CENT DRIED BLOOD

SOIL.	MG. OF NITRIC NITROGEN PRODUCED IN 21 DAYS		
	Directly after leaching	After crop crimson clover	After crop barley
Native	40 6	56 3	53 2
Synthetic alkali	1 4	33 5	23 8
Synthetic alkali, leached	46 9	64 7	87 2
Chloride	4 9	2 8	7 0
Chloride, leached	2 1	13 3	7 7
Sulphate	9 1	17 5	16 0
Sulphate, leached	5 9	10 9	19 3

removed and as the physical properties of the soil improve, the bacterial activities increase. On the other hand, leaching of the natural alkali soil did not restore its nitrifying powers, as few more nitrates were produced in the leached than in the unleached soil. It is in cases such as this that soil inoculation is essential to restore the natural microflora.

Soluble salts especially influence nitrogen fixation, and all evidence indicates that this class of microorganisms are more resistant to soluble salts than are the other beneficial bacteria.⁴

The alkali-containing soil uniformly gained in nitrogen when incubated with a soluble carbohydrate. The extent to which this occurs depends upon the specific salt present. It is most active in the presence of sulphate and least active in the presence of chlorides. Good *azotobacter* membranes invariably appeared when the sulphate-containing soils were used as the inoculant. The synthetic alkali soil was much more active in fixing nitrogen both before and

⁴ GREAVES, J. E., The microflora and the productivity of leached and non-leached alkali soil. *Soil Sci.* 23:271-302. 1927.

after leaching than was the native soil. When these soils were inoculated into Ashby media the nitrogen gains were even greater than they were in soil; consequently all results indicated that the alkali salts, if not present in too great concentration, accelerated nitrogen gains in soil. In order to obtain a more definite measure of the soil gains in nitrogen, they were carefully analyzed at the beginning of the experiment and at the end of two years.

TABLE IV

MILLIGRAMS OF NITROGEN FIXED IN 21 DAYS IN VARIOUS ALKALI
SOILS CONTAINING 1.5 PER CENT LACTOSE

SOIL	MG. OF NITROGEN FIXED IN 21 DAYS IN SOIL		
	Directly after leaching	After crop crimson clover	After crop barley
Native	— 6 3	— 0 8	10 5
Synthetic alkali	2 8	2 8	0 7
Synthetic alkali, leached	10 0	3 3	1 1
Chloride	4 1	0 9	1 8
Chloride, leached	0 0	1 8	0 3
Sulphate		3 9	8 3
Sulphate, leached		6 1	4 2

TABLE V

CALCULATED POUNDS OF NITROGEN GAINED BY 1 ACRE-FOOT OF SOIL DURING 2 YEARS

SOIL	DIRECTLY AFTER LEACHING (LB. PER ACRE)	END OF 2 YEARS (LB. PER ACRE)	TOTAL LOSS OR GAIN
Native	5397	4885	— 512
Synthetic alkali	4536	4863	327
Synthetic alkali, leached	4385	5055	670
Chloride	4738	5073	335
Chloride, leached	4334	5104	770
Sulphate	10,764	11,052	298
Sulphate, leached	10,300	11,442	1142

These soils were kept in the greenhouse with an optimum moisture content. The water from the city mains was used: this is low in soluble constituents. There had been produced on these soils one crop of crimson clover and two of barley. In the case of the alkali-containing soils the yield was light. Roots, stocks, and grain were removed when harvesting; hence little nitrogen could have come from the legume crop, yet the alkali-containing soils invariably registered appreciable gains of nitrogen. In the case of the leached

soils this was considerable. The sulphate-containing soil made the greatest gains. There was an annual loss of nitrogen from the check soil. The synthetic alkali soil gained annually 163 pounds of nitrogen. The gain in the leached soil was twice this amount. The chloride-containing soil gained 167 pounds of nitrogen per acre annually, with a gain of twice this amount by the leached soil. The sulphate-carrying soil gained annually 150 pounds of nitrogen, with a gain of four times this amount by the leached alkali soil. It is evident, therefore, that the alkali salts are increasing the nitrogen-fixing powers of these soils. The question at this stage was: what organisms are there within the soil responsible for this gain in soil nitrogen?

After the second crop of barley was harvested the junior author sampled the three leached soils and made a careful study of their specific microflora. The soils were first plated on nutrient, synthetic, and Ashby agar. There was a wide variation in the number and kind of colonies which developed on these different media.

TABLE VI

COLONIES DEVELOPING ON DIFFERENT MEDIA FROM LEACHED ALKALI SOIL

SOIL	NUTRIENT AGAR	SYNTHETIC AGAR	ASHBY AGAR
Synthetic alkali, leached	4,505,000	4,567,000	2,808,000
Chloride, leached	938,000	450,000	5,350,000
Sulphate, leached	5,600,000	3,878,000	3,855,000

The number of colonies developing varied with the media and soil. The synthetic alkali soil yielded approximately the same number of colonies on the nutrient and synthetic agar, with fewer on the Ashby. The chloride-carrying soil yielded many more colonies on the Ashby than on either of the others, whereas the sulphate-carrying soil yielded the greatest number on the nutrient, and fewer but approximately the same number on synthetic and Ashby agar. It was evident, therefore, that these soils contained many microorganisms which would develop on a nitrogen-poor medium such as Ashby's. The different colony types developing on Ashby agar were obtained in pure culture by repeated dilution, plating, and microscopic examination. They were then studied in detail morphologically and physiologically, according to the Official Methods.

Sixty-eight organisms were obtained in pure culture, all but 7 of which were gram-positive. There were 39 bacilli, 16 cocci, and 13 filamentous. Spores were produced by approximately 50 per cent, while only one-fourth of the organisms were motile. Glucose was fermented by 37, and of these 8 fermented lactose and 15 sucrose. All but 14 hydrolized starch. Ninety per cent produced ammonia when grown in a 1 per cent peptone solution. The quantity produced varied from 1 to 38 mg. Six produced over 20 mg. and one produced between 10 and 20 mg. Thirty-eight fixed nitrogen when incubated for 6 weeks in sterilized soil. The quantity fixed varied from 1.4 to 7 mg. of nitrogen in 100 gm. of soil. Eight organisms fixed over 5 mg. of nitrogen; consequently they compare favorably with the azotobacter in their nitrogen-fixing abilities. To date, however, it has been impossible to grow the organism and get appreciable nitrogen gains in nitrogen-free synthetic media.

Summary

The bacterial activities of soils are greatly modified by soluble salts. The numbers of bacteria ammonifying and nitrifying powers of the "alkali soils" tested are not fully restored by mere leaching of the soil. The extent to which restoration takes place varies with the concentration and kind of salt present. With the soils tested it was found that leaching is less effective in the presence of chlorides than it is in the presence of sulphates. The leached alkali soils studied rapidly fix nitrogen when seeded into Ashby media, when incubated with a soluble carbohydrate, or when kept under optimum moisture and temperature conditions in the greenhouse. In promoting nitrogen-fixation, the sulphates were found to be the most effective of the salts tested. Fifty-six per cent of the organisms obtained in pure culture from Ashby agar fixed nitrogen in soils containing mannitol, some of them fixing quantities which compare favorably with those fixed by azotobacter. The beneficial bacteria of the soil survive for long periods in soils containing comparatively high concentrations of chlorides and sulphates.

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CURRENT LITERATURE

BOOK REVIEWS

Weeds in agriculture

The subject of weeds in agriculture has received by no means the scientific and fundamental study which it deserves, and which has been devoted to many other phases of agricultural science. The problem of weeds is of extreme importance, although they are often accepted as inevitable. The weed books of the world, with the exception of a few excellent monographs, are in the main ancient compilations, inadequate, and not the kind of literature to impress farmers favorably with the efficacy of the scientific weed expert.

The publication of KORSMO's monumental work,¹ therefore, is an event of importance. It is translated by WOLLENWEBER into German from the original Norwegian. This translation into German is a step in the right direction, although an edition in English would give the volume a still broader availability.

The work incorporates the results of 35 years' fundamental research, and easily surpasses in valuable detail, original thought, and practical suggestions anything so far published, to the reviewer's knowledge, in any other language. KORSMO's effort has produced an opportune standard work on weeds which is certain to earn for itself first place in the scientific literature of agriculture in any country.

The material is divided into eight main sections including divisions of weeds into biological groups; the occurrence of weeds under agricultural practices; the injurious influence of weeds by crowding out of crop plants, or through overshadowing; etc. A section is devoted to a discussion of propagation and dispersal, followed by a section dealing with living weed seeds and perpetuation through underground portions of weeds in arable land. Another section deals in detail with the different kinds of weeds: "About 8-10 per cent of the entire vegetation in countries of the northern temperate zone consists of weeds," it is stated. A most important section is devoted to preventive and control measures. Chemical warfare against weeds is very thoroughly dealt with.

The following section summarizes the results of a number of Norwegian experiments on the control of weeds during crop growth, actually carrying into

¹ KORSMO, EMIL, *Unkräuter im Ackerbau der Neuzeit, biologische und praktische Untersuchungen*. (Weeds in agriculture of modern times, biological and practical investigations.) Translated into German from the Norwegian manuscript by H. W. WOLLENWEBER. pp. 580. figs. 470. Berlin: Julius Springer. 1930.

practice the recommendation of section 6 B, III and IV, which afford striking proof of the exceedingly beneficial effects of proper weed control measures.

The final section reviews the whole subject. One series of experiments indicates the practical results from rational weed control. From 854 experiments with cereals, an average increase in yield through weed control of 490Ko. grain and 365Ko. straw per approximately two acres was gained. Similarly in 70 root crop experiments the gain was 9313Ko. of roots and 1916Ko. of tops, while in 102 experiments with potatoes on the average an increase of 4237Ko. of tubers was obtained per hectare (1 acre=0.476 hectare). Cost of control measures, etc., having been carefully calculated and taken into consideration, the author states: "From all this may be deducted with great clearness the fact that the practice of weed control measures pays the farmer."

An instructive world bibliography on weeds is followed by an exhaustive index.

KORSMO has rendered an inestimable service to world agriculture by the publication of his research data. The world owes KORSMO in return a war against weeds, aided by international legislation to prevent further distribution in these days of rapid transit and intimate intercourse, if such measure is not already too late.—H. T. GÜSSOW.

Use of the microscope

A volume² has just appeared which is designed to show in detail how to secure the best results with the microscope under all circumstances. It is a study of the combination of adjustments and variations of methods which will add to the perfection of the image in the microscope. The most important of these methods have been brought together from the original literature, and the author has added the results of his own experience.

The introduction lists 62 causes of injury to the microscopical image and the preventives to be employed in avoiding them. There are 26 chapters which treat of such subjects as different types of microscopes, types or kinds of objectives, oculars and condensers, illumination, light filters and screens, the cover glass problem, drawing, photography, care of the microscope, and others. It has a short chapter on fixing and staining microscopical objects, another which lists a hundred microscopical objects of biological interest, and another which gives fifty practical exercises with the microscope.—J. M. BEAL.

² BELLING, J., *The use of the microscope*. 8vo. pp. xi+315. figs. 28. New York: McGraw-Hill Book Co. 1930. \$4.

THE BOTANICAL GAZETTE

November 1930

ASPEN ASSOCIATION IN NORTHERN LOWER MICHIGAN¹

FRANK C. GATES

(WITH FOURTEEN FIGURES)

Introduction

The aspen association is by far the most important secondary association in northern Michigan, revegetating nearly every type of location following the removal of virgin growth, thus standing between the devastated land and development of the most desirable trees, whether for lumber forests or for recreational purposes. The importance of an understanding of the aspen association is paramount from either of these considerations, since so much of northern Michigan is suitable economically for the most part only for raising trees. An ecological study in the area should contribute to such an understanding.

The field work upon which this paper is based was done in Emmet and Cheboygan counties at the northern end of the lower peninsula of Michigan between 1911 and 1929, while the writer was on the staff of the Biological Station of the University of Michigan on Douglas Lake (2, 6).

Aspen association

From a distance the general appearance of the aspen association in this region is that of light green patchy crowns forming an irregu-

¹ Contribution from the Biological Station of the University of Michigan, and no. 285 from the Department of Botany, Kansas State Agricultural College, Manhattan, Kansas.

lar skyline. If there are relic trees (figs. 1, 2) of former associations, these are the highest to be seen; if not, the aspens form the upper story at a height of about 2-10 m. or more. Under the trees and in the open spaces between there may be a layer of higher shrubs (about 1-2 m.), a layer of bracken ferns 0.5-0.8 m. high (fig. 3), a slightly lower layer of *Diervilla*, and a ground layer of herbaceous or somewhat woody plants among which certain grasses are often abundant, or open patches of *Cladonia* or *Polytrichum*. Except sometimes for a year or two under the canopy of the multitudinous aspen sprouts of a



FIG. 1.—Sandy upland aspens showing many relic pines (*Pinus resinosa* and *P. strobus*); June 25, 1915.

recently burnt-over aspen area, the shade of aspen trees, even in the densest places, permits the development of large shade forms rather than excluding ground vegetation. A dense ground cover of partially decayed leaves is frequent in lowland areas. In young areas dead branches bristle from the trunks, but before long these are self-pruned. Rotting logs of former associations may be strewn about, but dead aspen branches or trunks decay quickly on reaching the ground.

Comparing the principal consociates, the one dominated by *Populus grandidentata* is the most open and has the lightest green color; that dominated by *P. tremuloides* has the tallest trees; and that dominated by *Prunus pennsylvanica* is the densest.

Habitats and factor relationships

In general a wide range of habitats may be occupied by the aspen association, from the very driest in the region to very wet although not persistently submerged soil, and from almost pure sand to a



FIG. 2



FIG. 3

FIGS. 2, 3.—Fig. 2, sandy upland aspens north of Burt Lake, showing groups of pines overtopping *Populus grandidentata*; August 13, 1916; fig. 3, interior view of sandy upland aspens in which pines are beginning to replace *Populus grandidentata*; ground cover of *Pteris aquilina* in evidence; August 12, 1922.

somewhat heavy clayey soil. The pH values obtained at various times are on the acid side of neutrality, between 4.6 and 6.6. In this region the association is nearly independent of topographic features, which, however, are not very diverse. With their rather extensive although not deeply penetrating root system, the trees are able to stand considerable drought during the summer. Evaporation may exceed 65 cc. per day, although it is usually less than 10 cc. Excessively high temperatures occasionally occur (3, 5), but only rarely is the heat sufficient to desiccate, burn, or kill aspen plants. In 1917, temperatures of 52° C. on ground debris and 60° on open sand were recorded (8). The highest temperature I have recorded on open sand in the aspens is 66° C. The mineral soils following fires, especially those with a surplus of potassium (12), are favorable to the development of the aspen association. Aspen seedlings frequently appear in sandy areas where water or ice work prevents their developing into trees; otherwise no ordinary features of land habitat exclude aspens.

INCEPTION OF ASSOCIATION

It is not known just when the aspen association originally made its appearance in this region. The supposition is that fires were occasional, set by Indians or by lightning, so that even before the advent of white men there were patches of the aspen association. At the present time the inception of the association in a given area is the following. An area of virgin or second growth forest is burnt over. A year or two after the fire, fireweeds appear. This development takes place soonest on lowland unless the spring is favorably wet. The most prominent fireweed in this region is *Epilobium angustifolium* (fig. 4), although *Erigeron canadensis* and *Galeopsis tetrahit* are sometimes abundant. With them there may be a few aspen seedlings, but these may be so completely overshadowed by the vigorous development of the fireweeds as to lose existence. In the following years such fireweed plants as appear are less dense, more reduced in stature, and consequently offer little or no resistance to aspen colonization. New aspen seedlings or former ones then develop. A year or two after a fire on very low land, *Marchantia* may appear with a few mosses (*Polytrichum* spp. and *Ceratodon purpureus*) and fireweeds. The latter, especially *Epilobium angusti-*

folium, increase greatly during the next year or two. Aspens then have their best chance for establishment. On the better soils the sprouting of hardwood stumps may prevent the inception of an aspen association in favor of a hardwood coppice, or, with additional fires, brambles. Under the latter circumstances if the brambles are at all dense additional fires are necessary to give the aspens a chance to develop.

For the natural development of a well developed aspen area in which the aspen dominants form more than 95 per cent of the trees,



FIG. 4.—Former beech-maple forest west of Douglas Lake entirely covered with *Epilobium angustifolium*; July 19, 1915.

additional fires must occur, but not at too short intervals. Such repeated fires are usually favorable, as the aspens sprout readily from the stumps or underground parts. The pines of this region cannot sprout nor can the normal bog trees. The maples and beeches can, but sprouting is slower than in the aspens.

ECOLOGY OF ASSOCIATION

The association consists of several dominant species and a large number of secondary species. Only a few of the latter are truly characteristic. Most of them are normal in other associations of similar ground, but can still grow under the conditions presented by

the aspen association. Of 310 species of seed plants found in the association, 22.9 per cent are phanerophytes, 3.9 per cent are chamaephytes, 47.1 per cent are hemicryptophytes, 16.1 per cent are geophytes, and 10.3 per cent are therophytes. This marked ascendancy of hemicryptophytes over normal is what one would expect in a region with a climate of considerable rigor in the unfavorable season.²

Certain correlations of soil, water, and species make it possible to recognize three types or consocieties within the aspen association: lowland, sandy upland, and clayey upland. Each is distinguished by the particular abundance of one of the codominant species, as shown in table I.

TABLE I
SOIL TYPE AND PERCENTAGE OF PRINCIPAL TREES

SOIL	DOMINANT SPECIES	PERCENTAGE OF TREES			
		<i>Populus grandidentata</i>	<i>Populus tremuloides</i>	<i>Prunus pennsylvanica</i>	<i>Betula papyrifera</i>
Sandy upland. . . .	<i>Populus grandidentata</i>	50	10	5	20
Sandy lowland. . . .	<i>Populus tremuloides</i>	5	60	2	12
Clayey upland. . . .	<i>Prunus pennsylvanica</i>	4	1	60	14

Populus grandidentata (figs. 3, 5).—This species is most abundant in the drier sandy areas on which the aspen association occurs. The root system is widespread rather than deeply penetrating, which is somewhat favorable to windfall. Growth is rapid at first but within a few years decreases remarkably. Flowering and the maturing of seeds occur early in the growing season. The seeds are wind-distributed and lose vitality rapidly. Trees from seeds or stump sprouts are soon thinned out on account of their intolerance, resulting in an open woodland. Following fire the stumps and underground parts sprout vigorously (fig. 6). As many as 20 sprouts have been counted from a single stump the first year after a fire. Under the most favorable circumstances a sprout 3.68 m. has arisen, but normally they do not exceed 1–2 m. in the first year. In the course of 3 or 4 years

² ENNIS, BEULAH, The life forms of Connecticut plants and their significance in relation to climate. Bull. Conn. Geol. & Nat. Hist. Surv. 43. 1928.

most of these sprouts die, usually leaving one or two to develop into trees. The leaves of these sprouts are sometimes enormous in size



FIG. 5.—Interior of well developed *Populus grandidentata* consociates showing dominant species and layer of *Pteris aquilina* (photograph by G. E. NICHOLS).



FIG. 6.—Former dense sandy upland aspen area burned in May, 1921, showing two seasons' regeneration of *Populus grandidentata*; August 12, 1922.

and differ markedly from normal leaves. Insects apparently do not trouble the aspens of this region very much. The aspen borer (*Saperda calcarata* Say.) is present, but its depredations are mostly on trimmed trees near the lake. Old trees usually have heart rots, but many die when about 20–25 years old from no apparent cause. ROBINOVE and HORTON (16) state: "The largest tree of *P. grandidentata* found was 12.5 m. high, 35 years old and had a diameter of 18.5 cm., but the top was dead and the trunk badly heart rotted." Two others were a little older and thicker, but 2.75 m. shorter and more rotten.



FIG. 7.—Bogland aspens near Maple Point dating from 1914 showing *Populus tremuloides* (road cut in 1924); July 14, 1925.

Populus tremuloides (fig. 7).—In general appearance this is somewhat similar to the preceding species, but differs in being a more compact tree, preferring wetter areas, although it does grow in just as sandy land. It grows to a larger size. Its ability to develop in burnt-over bogs where it frequently occurs in nearly pure stands marks it as somewhat different ecologically from the large-toothed aspen. These trees grow closer together than *P. grandidentata*, because their leaves are smaller, transpire slower, and are nearer the trunk. The shade cast by it when growing in a dense thicket may be sufficient to preclude the development of much ground vegetation.

The largest trees date back to the time of the original lumbering in the region. ROBINOVÉ and HORTON (16) state: "The two largest trees of *P. tremuloides* found were both 20.5 meters high and 33.5 cm. D.B.H., 62 years old, and 35.5 cm. D.B.H., 59 years old respectively."

Populus balsamifera.³—In some lowland areas this species is associated with *P. tremuloides*. There are few such cases, but west of Lancaster Lake the few balsam poplar trees are as tall as the highest *P. tremuloides* of the same age, thicker in diameter, and are also



FIG. 8.—Beech-maple forestland occupied by *Prunus pennsylvanica* consociates of aspen association; July 9, 1919.

sound trees. "The largest was a sound tree, 62 years old, 40.6 cm. in diameter and 21.5 meters high" (16).

Prunus pennsylvanica (fig. 8).—This is likewise a small tree, but rather more branching than either aspen. Its leaves are longer, much narrower, and produced in greater profusion. The bark is bright brown or reddish brown. Otherwise there are many similarities among them. An important difference is its decided preference for heavier soils, containing clay, characteristic of land on which the

³ The scientific names employed are those of the standard manuals (BRITTON and BROWN, 2d. ed; GRAY, 7th ed) except that the U.S. Department of Agriculture is followed with respect to grasses (HITCHCOCK, Bull. 772) and with respect to forest trees (SUDWORTH, Circ. 92).

beech-maple forest had developed. Consequently, on the better soils the aspen association is usually represented by the *P. pennsylvanica* consociates. While this species will grow in sandy ground, unless the vegetation is very open it is not likely to be an important factor in aspen development. It is more resistant to fire, but if burned will sprout vigorously and even to better advantage than the aspens. In so doing it may develop into an important tree in aspen areas on sandy ground. The tree is subject to occasional insect attack. Tent caterpillars at times retard its growth. It is much less subject to windfall as it is more deeply rooted. It is not as intolerant, consequently not so thoroughly self-pruned. As the dry twigs are far from brittle a dense mass is present even in rather high thickets. The fruit is eaten by birds. The various seeds such birds leave under the trees enrich the aspen flora.

Betula papyrifera.—This is a conspicuous tree in the aspens. Its general characteristics are closer to those of *P. pennsylvanica* than they are to the real aspens. Its preference is for proximity to lakes, where moist winds temper the summer dry spells, thus in a measure preventing the too severe over-heating of the tree trunk, which it cannot withstand, having a nearly waterproof bark. The birch may be present on either sandy or clay land with no special preference, so far as the data from this region indicate. The trees are rather deeply rooted, so that while windfall is occasional it is not so frequent as in the aspens. Following fire the stumps sprout vigorously. More of the sprouts produce trees than is the case with the species of *Populus*. If the birches are growing close together they may reach a moderate height. Some such trees are about 17 m. high, with a diameter of about 20 cm., but if cutting is done around them, so that the trunks are exposed during the summer they generally die, or at best make very slow growth. A few areas have an entire dominance of birch, but more usually these are mixed with species of *Populus*.

SUBDOMINANT SPECIES

There are two species which are abundant and characteristic of the aspens, whether or not the trees are present. It is necessary to consider them as subdominants, since they may act as dominants both in invading new areas and in remaining as relics if the aspen

is replaced. They occur in great abundance in the aspens themselves, often composing 85–90 per cent of the ground vegetation. Of these, *Pteris aquilina* (figs. 3, 5, 9) has rhizomes about 20–25 cm. below the surface of the ground, out of reach of fire. While underground parts as deep as this might be destroyed in the burning of the original forest, by the time *Pteris* is established in the areas there is not enough combustible material on the surface to heat to the death point so far below the surface. The abundance and vigorous growth



FIG. 9.—Edge of former beech-maple area near Mud Lake cultivated through 1914; abandonment followed by development of bluegrass meadow into which *Pteris aquilina* is rapidly spreading from adjacent *Populus tremuloides* consociates (eleven years later meadow was completely replaced by *Pteris*); August 14, 1917.

of the rhizomes make *Pteris* a most important contender for space. In a former cultivated patch, now grass, *Pteris* has been advancing about 2 m. a year (fig. 9). The leaves spread out at a height of 30–50 cm. in the sun, or in a shaded area as high as 1.3 m. They cover the ground with a moderate shade, which, however, does not preclude the development of various secondary plants. In the deeper aspen thickets only very pronounced shade-loving forms can stand the combined shade of aspens and a dense *Pteris* growth. Only in abnormally dry or hot summers is *Pteris* visibly affected by drought (8). In an extremely hot spell in July, 1917, and in the hot summer

of 1921, many leaves sunburnt, wilted, and dried up, producing an effect similar to that of late killing frosts.

Diervilla diervilla is the second subdominant species in the aspens. It is a low shrub, chamaephyte-nanophanerophyte, usually not over 30 cm. high, generally dying down to near the ground in winter. It is decidedly abundant in many places, sometimes carpeting the ground to the exclusion of almost all other vegetation. While it

TABLE II
PERCENTAGE FREQUENCY OF TREES AND HIGH SHRUBS IN ASPENS*

NAME	PINELAND	HARDWOOD OR BEECH-MAPLE	LOWLAND OR BOG	ECOLOGICAL CLASSIFICATION
<i>Abies balsamea</i>	0 01	**	i
<i>Acer pennsylvanicum</i>	0 12	1 12	*	s
<i>Acer rubrum</i>	7.18	6 73	***	s
<i>Acer saccharum</i>	0 18	4.49	*	r, i
<i>Acer spicatum</i>	0.002	0.04	**	s
<i>Alnus incana</i>	0 55	**	s
<i>Betula papyrifera</i>	17 98	13 99	***	d
<i>Fagus grandifolia</i>	0 70	5 37	*	r, i
<i>Fraxinus nigra</i>	**	i
<i>Pinus resinosa</i>	1 36	**	r, i
<i>Pinus strobus</i>	1 69	**	r, i
<i>Populus grandidentata</i>	49 37	3.26	**	d
<i>Populus tremuloides</i>	10 40	0 93	****	d
<i>Prunus pennsylvanica</i>	3 69	57 62	**	d
<i>Quercus borealis</i>	2.96	0 50	*	s, i
<i>Rhus glabra borealis</i>	1 03	2 86	s
<i>Salix bebbiana</i>	1.27	1.19	**	s
<i>Sambucus racemosa</i>	0.002	0 98	*	s
<i>Thuja occidentalis</i>	0 003	**	r, i
<i>Tsuga canadensis</i>	0.02	*	i

* The percentage frequency or frequency index in pineland is based on 12 years' statistical data and that of hardwoodland on 7 years. In the case of lowlands, successional changes so complicated the stations originally chosen, that asterisks are used to indicate the approximate frequency: one for less than 1%, two for 1-5%, three for 6-10%, and four for over 10%. The ecological classification in this and subsequent tables applies to the whole aspen association: d indicates dominant species; s, secondary species, r, relic species, and i, invading species.

develops better in some shade, adequate water, and in richer soil, it may proceed out on to open sand in advance of the aspen trees. Under such conditions it develops anthocyanin in its leaves, giving a characteristic red color which differs from that of autumn coloring or a dying or improperly functioning leaf. Experiments (KEENER 13) have shown that the green color may be restored by shading, enriching the soil, or by proper watering.

Associated species (general).—In the aspens the number of associated species is great and their distribution through the area

TABLE III

FREQUENCY INDEX OF MORE IMPORTANT GROUND PLANTS IN
ASPEN ASSOCIATION*

NAME	PINELAND	HARDWOOD OR BEECH-MAPLE	LOWLAND OR BOG	ECOLOGICAL CLASSIFICATION
<i>Abies balsamea</i>	0 02	**	i
<i>Acer pennsylvanicum</i>	0.23	2.94	*	i
<i>A. rubrum</i>	7.65	9 93	****	s
<i>A. saccharum</i>	0 05	5 16	*	i
<i>A. spicatum</i>	***	i
<i>Achillea millefolium</i>	0.06	0.95	*	s
<i>Agrostis hyemalis</i>	2 66	15 42	*	s
<i>A. palustris</i>	1.83	8 27	*	s
<i>Alnus incana</i>	0 07	**	i
<i>Amelanchier canadensis</i>	0.99	0 08	*	s
<i>Anaphalis margaritacea</i>	0 71	9.79	*	s
<i>Antennaria canadensis</i>	2 12	1 72	*	s
<i>Apocynum androsaemifolium</i>	5 51	6 12	***	s
<i>Arabis glabra</i>	0.33	3 30	s
<i>Aralia hispida</i>	0.31	13.15	*	s
<i>A. nudicaulis</i>	3.24	1 31	****	s, r
<i>Arctostaphylos uva-ursi</i>	0 28	*	r
<i>Asclepias syriaca</i>	0 53	2 28	s
<i>Asplenium filix-femina</i>	***	r
<i>Aster laevis</i>	26 80	6 15	***	s
<i>A. macrophyllus</i>	2 43	0 17	**	r, s
<i>Betula papyrifera</i>	6 70	4 42	***	d
<i>Botrychium virginianum</i>	0 08	***	r
<i>Brachyelytrum erectum</i>	**	s
<i>Carex communis</i>	0 14	6 78	s
<i>C. gracillima</i>	**	r
<i>C. intumescens</i>	0 02	0 07	**	r
<i>C. umbellata</i>	18 45	6 04	**	s
<i>Chimaphila umbellata</i>	1.84	0 30	*	r
<i>Cirsium arvense</i>	2.34
<i>Clintonia borealis</i>	1 03	0.34	**	r
<i>Comandra umbellata</i>	5.97	0 08	***	s
<i>Convolvulus spithameus</i>	12.94	0.13	s
<i>Cornus alternifolia</i>	**	s
<i>C. canadensis</i>	1.33	0.54	***	r
<i>C. stolonifera</i>	0 05	**	r
<i>Corylus rostrata</i>	0 01	**	s
<i>Danthonia spicata</i>	7.57	**	s
<i>Diervilla diervilla</i>	41.82	24 44	****	sub-d
<i>Dryopteris spinulosa</i>	**	r
<i>D. thelypteris</i>	0.18	**	r
<i>Epilobium angustifolium</i>	2.83	21.86	****	r, s
<i>Equisetum arvense</i>	0.19	***	s
<i>E. sylvaticum</i>	0 21	****	r
<i>Erigeron canadensis</i>	2.48	8.72	**	r, s
<i>E. philadelphicus</i>	1.02	0.12	**	s
<i>E. ramosus</i>	1.12	s
<i>Fagus grandifolia</i>	1.09	11.52	*	i
<i>Fragaria virginiana</i>	5.08	2 83	***	s

* See footnote to table II.

TABLE III—Continued

NAME	PINELAND	HARDWOOD OR BEECH-MAPLE	LOWLAND OR BOG	ECOLOGICAL CLASSIFICATION
<i>Fraxinus nigra</i>			**	i
<i>Galium circaezans</i>			**	r, s
<i>G. trifidum</i>	0.10		**	r, s
<i>G. triflorum</i>	0.07		***	r, s
<i>Gaultheria procumbens</i>	46 08	3 82	****	r, s
<i>Gaylussacia baccata</i>	2 97		**	r
<i>Gnaphalium decurrens</i>	2 91	15.02	*	s
<i>Hieracium scabrum</i>	3.89	0 77	***	s
<i>H. venosum</i>	6.34	0 27	*	s
<i>Lactuca canadensis</i>	7.24	30 35	****	s
<i>L. sagittifolia</i>	0 64	6.24	**	s
<i>Lepidium virginicum</i>	0 92	1 41	**	s
<i>Lonicera canadensis</i>	0.20	0 08	**	s
<i>L. dioica</i>	0 15	0 12	*	s
<i>Melampyrum lineare</i>	12 74	0 25	****	s
<i>Mitchella repens</i>	0 55	0 37	**	r
<i>Mitella nuda</i>			*	r
Moss and lichen cover	26 37	39 37	***	
<i>Onoclea sensibilis</i>	0 02		**	r
<i>Oryzopsis asperifolia</i>	7 64	0 29	**	s
<i>O. pungens</i>	3 30	0 06	*	s
<i>Panicum depauperatum</i>	1 81			s
<i>P. meridionale</i>	3 73	0.53	*	s
<i>P. xanthophyllum</i>	2 75			s
<i>Pedicularis canadensis</i>	3 18		*	r, s
<i>Phleum pratense</i>	0 24	7 70	**	s
<i>Pinus resinosa</i>	1.50		**	i
<i>P. strobus</i>	4 23		**	i
<i>Poa compressa</i>	2 63	7.24	*	s
<i>P. palustris</i>	0 49	2 09	**	s
<i>P. pratensis</i>	10 81	71 96	**	s
<i>Polygala paucifolia</i>	1 76		**	r, s
<i>Polygonatum biflorum</i>	0 21	0 87	*	r
<i>Populus grandidentata</i>	15 15	1 45	*	d
<i>P. tremuloides</i>	11 15	2 38	***	d
<i>Prunus pennsylvanica</i>	6.03	33 80	*	d
<i>Pteris aquilina</i>	92 51	35.11	****	sub-d
<i>Pyrola elliptica</i>	0.27	0.12	***	r
<i>P. secunda</i>	0.29		***	r
<i>Quercus borealis</i>	3 81	0 48	*	i
<i>Rhus glabra borealis</i>	17 97	21 80		s
<i>R. toxicodendron</i>	0 35		*	s
<i>Ribes triste</i>		0.48	**	r
<i>Rosa blanda</i>	2 05		*	s
<i>Rubus allegheniensis</i>	24.08	17.22	****	s
<i>R. strigosus</i>	0.32	50.77	**	s
<i>R. triflorus</i>	0.29		****	s
<i>Rumex acetosella</i>	8.45	10.25		s
<i>Salix bebbiana</i>	3.74	3.99	***	s
<i>S. discolor</i>	0 19	0.18	**	r
<i>S. discolor</i> × <i>bebbiana</i>	0.10		*	s
<i>Sambucus racemosa</i>	0.02	7.84	*	r
<i>Solidago canadensis</i>	3.41	18.26	****	s

TABLE III—*Continued*

NAME	PINELAND	HARDWOOD OR BEECH-MAPLE	LOWLAND OR BOG	ECOLOGICAL CLASSIFICATION
<i>S. hispida</i>	21.84	2.34	***	s
<i>Taraxacum vulgare</i>	1.34	10.88	***	s
<i>Taxus canadensis</i>	0.05	**	r
<i>Thuja occidentalis</i>	***	i
<i>Trientalis americana</i>	1.22	0.45	***	r, s
<i>Trifolium pratense</i>	0.03	*	s
<i>T. repens</i>	0.23	0.12	**	s
<i>Trillium grandiflorum</i>	0.04	5.27	**	r
<i>Tsuga canadensis</i>	0.20	0*	*	i
<i>Unifolium canadense</i>	7.61	5.44	****	r, s
<i>Vaccinium angustifolium</i>	20.87	0.51	***	r, s
<i>V. canadense</i>	4.58	0.23	*	r, s
<i>V. nigrum</i>	1.81	*	r, s
<i>Vagnera racemosa</i> ...	2.36	3.35	***	r
<i>V. stellata</i>	0.01	0.06	**	r
<i>Verbascum thapsus</i> ..	3.36	12.38	*	s
<i>Viola canadensis</i>	***	s
<i>V. eriocarpa</i>	0.02	**	r
<i>V. pallens</i>	0.13	**	r
<i>V. papilionacea</i>	0.06	**	r

* As an invader in a few areas not studied with quadrats.

irregular, both with reference to the exact conditions of habitat and the general local conditions. The great frequency of a relatively limited number of ground plants, however, makes the frequency index community coefficient (10) between stations quite high, whether adjacent or separated by a few kilometers. Within the same kinds of sites, the frequency index community coefficient is usually above 60 and frequently above 90. Even when comparing sets on bogland and sandy uplands coefficients of 30 are obtained. The origin of the associated species is manifold. Some of them are characteristic of aspens, while others belong more properly to other associations. Briefly, almost any plant that is capable of growing in such an area is likely to be found if sought for carefully, but only a few have high frequency indices.

While several types of secondary species are present (tables II and III), the majority belong to types that are less than 50 cm. high. Except where bluegrass may form a sod, there is usually much bare ground visible. Great patches of ground may be covered with certain mosses (*Polytrichum* spp.) or lichens (*Cladonia rangiferina* and others).

Seasonal aspects are not nearly so striking as those in either the prairie or the jackpine forests. A few such outstanding plants are:



FIG. 10.—Details of *Convolvulus spithameus*, common sandy upland aspen plant (photograph by C. W. HORTON).



FIG. 11.—*Lycopodium tristachyum* in sandy upland aspens near Douglas Lake; July 25, 1926.

in early spring, *Antennaria canadensis*, *Senecio pauperculus*, *Vaccinium* spp.; in early summer, *Pedicularis canadensis*, *Panicum xanthophyllum*, *Comandra umbellata*; in midsummer, *Melampyrum lineare*, *Hieracium venosum*, *Convolvulus spithameus* (fig. 10), *Lycopodium tristachyum* (fig. 11), *Rhus glabra*; and in late summer and autumn, *Aster laevis*, *Solidago hispida*, and *S. canadensis*.

ADDITIONAL TREES AND HIGH SHRUBS IN ASPENS WITH
PERCENTAGE FREQUENCY IN ALL CASES BELOW 1%

<i>Amelanchier canadensis</i> (P,* h, b, s)	<i>Ostrya virginiana</i> (h, b, i)
<i>A. sanguinea</i> (p, s)	<i>Picea glauca</i> (p, b, i)
<i>A. spicata</i> (p, h, s)	<i>P. mariana</i> (p, i)
<i>Aronia arbutifolia</i> (p, s)	<i>Pinus banksiana</i> (p, i)
<i>Betula lutea</i> (p, H, B, i)	<i>Populus balsamifera</i> (P, b, d)
<i>Chamaedaphne calyculata</i> (b, r)	<i>Prunus nigra</i> (b, i)
<i>Cornus alternifolia</i> (p, B, s)	<i>P. serotina</i> (p, h, b, i)
<i>C. baileyi</i> (b, s)	<i>P. virginiana</i> (P, H, b, s)
<i>C. rugosa</i> (p, b, s)	<i>Salix discolor</i> (p, h, B, s, r)
<i>C. stolonifera</i> (p, B, s)	<i>S. discolor</i> × <i>bebbiana</i> (p, B, s, r)
<i>Corylus rostrata</i> (p, B, s)	<i>S. lucida</i> (p, h, b, s, r)
<i>Crataegus roanensis</i> (p, s)	<i>Sambucus canadensis</i> (h, s)
<i>Fraxinus americana</i> (p, H, B, r, i)	<i>Taxus canadensis</i> (p, b, s)
<i>Hamamelis virginiana</i> (p, s)	<i>Tilia glabra</i> (p, h, B, i)
<i>Larix laricina</i> (p, B, r, s, i)	<i>Ulmus americana</i> (p, B, i)
<i>Lonicera canadensis</i> (p, s)	<i>Viburnum acerifolium</i> (p, h, b, s)
<i>L. dioica</i> (p, s)	<i>V. cassinoides</i> (p, b, s)
<i>L. hirsuta</i> (b, s)	<i>V. lentago</i> (p, b, s)
<i>Nemopanthus mucronata</i> (p, r)	

ADDITIONAL GROUND PLANTS IN ASPENS WITH FREQUENCY
INDEX IN ALL CASES BELOW 1†

<i>Actaea rubra</i> (B, r)	<i>Anemone quinquefolia</i> (p, s)
<i>Adiantum pedatum</i> (h, r)	<i>Antennaria neodioica</i> (P, s)
<i>Agrimonia gryposepala</i> (p, h, B, r)	<i>A. plantaginifolia</i> (P, s)
<i>Agropyron repens</i> (P, H, B, s)	<i>Apocynum cannabinum</i> (p, s)
<i>Amaranthus graecizans</i> (H, s)	<i>A. medium</i> (p, s)
<i>Amelanchier sanguinea</i> (p, s)	<i>A. sibiricum</i> (p, H, s)
<i>A. spicata</i> (P, B, s)	<i>Aquilegia canadensis</i> (B, s)

* The letters P, H, B, in this list and in that following, indicate respectively presence in pineland, hardwoodland or beech-maple and lowland or bogland aspens. Lower case letters (p, h, b) indicate frequencies below 0.1%.

† In addition to these plants, 53 species have been found at least more than once in the aspens, but in this study have never happened to appear in any of the quadrats counted.

- Arabis brachycarpa* (P, H, s)
Aralia racemosa (B, s)
Arctium minus (p, s)
Asclepias exaltata (P, H, s)
Aster lateriflorus (b, r)
A. junceus (p, B, r)
A. tradescanti (p, B, r)
A. novae-angliae (p, H, B, r)
Betula lutea (H, B, i)
Botrychium simplex (B, r)
B. onandagense (b, r)
Bromus ciliatus (p, s)
Calamagrostis canadensis (p, b, r)
Caltha palustris (B, r)
Carex adusta (P, H, s)
C. aenea (P, h, s)
C. foenea (P, h, B, s)
C. laxiflora (p, H, B, r)
C. lucorum (p, s)
C. pennsylvanica (p, s)
C. stipata (p, B, r)
Cerastium vulgatum (p, s)
Chenopodium album (H, s)
C. capitatum (H, s)
Cinna latifolia (b, r)
Circaea alpina (B, r)
C. lutetiana (b, r)
Clinopodium vulgare (B, s)
Coptis trifolia (p, B, r)
Corallorrhiza maculata (B, r)
Cornus baileyi (B, r)
C. rugosa (P, B, s)
Crataegus roanensis (p, s)
Cypripedium acaule (P, h, B, s)
Dactylis glomerata (H, s)
Dryopteris marginalis (B, r)
Epigaea repens (P, r)
Epilobium adenocaulon (p, b, s)
Equisetum hyemale (p, s)
E. variegatum (p, B, r)
Erigeron annuus (p, s)
Eupatorium perfoliatum (H, r)
E. purpureum (B, r)
Festuca occidentalis (p, h, s)
F. ovina (p, s)
Filix bulbifera (H, B, r)
F. fragilis (B, r)
Fraxinus americana (p, H, B, i)
Geranium robertianum (p, h, r)
Geum rivale (B, r)
G. strictum (b, r)
Helianthemum canadense (p, s)
Helianthus tuberosus (H, B, s)
Hepatica triloba (p, h, r)
Hieracium aurantiacum (P, H, B, s)
H. canadense (p, s)
H. gronovii (P, s)
H. paniculatum (p, h, s)
Hypericum perforatum (P, s)
Hypopitys lanuginosa (p, s)
Hystrix hystrix (h, b, r)
Ilex verticillata (B, r)
Impatiens biflora (B, r)
Juncus effusus (b, r)
Juniperus communis depressa (p, r)
Lactuca scariola (P, H, s)
L. spicata (h, s)
Larix laricina (p, B, i)
Lilium philadelphicum andinum (b, r)
Linnaea borealis (P, B, r)
Lonicera hirsuta (p, B, s)
L. glaucescens (p, B, s)
Lycopodium annotinum (P, H, r)
L. obscurum (p, r)
L. tristachyum (P, h, B, s)
Lysimachia terrestris (B, r)
Medeola virginiana (H, b, r)
Melica purpurascens (h, b, r)
Needle and debris cover (P)
Nabalus racemosus (p, B, s)
Nemopanthus mucronata (p, b, r)
Oenothera biennis (p, s)
O. muricata (p, b, s)
Osmunda cinnamomea (B, r)
O. regalis (B, r)
Panicum implicatum (p, s)
P. linearifolium (P, s)
P. perlongum (P, s)

<i>P. tennesseense</i> (p, s)	<i>Scrophularia leporella</i> (p, s)
<i>Picea glauca</i> (p, B, i)	<i>Senecio pauperculus</i> (P, B, s)
<i>P. mariana</i> (b, i)	<i>Silene antirrhina</i> (p, s)
<i>Pinus banksiana</i> (p, i)	<i>Solidago graminifolia</i> (p, H, B, s)
<i>Petasites palmata</i> (P, b, r)	<i>S. juncea</i> (P, s)
<i>Poa saltuensis</i> (b, s)	<i>S. rugosa</i> (p, B, r)
<i>Polygonum convolvulus</i> (p, H, s)	<i>S. altissima</i> (b, r)
<i>P. persicaria</i> (p, B, s)	<i>Sorbus americana</i> (H, B, i)
<i>Populus balsamifera</i> (P, d)	<i>Streptopus amplexifolius</i> (B, r)
<i>Prunella vulgaris</i> (p, b, r)	<i>S. roseus</i> (H, B, r)
<i>Prunus cuneata</i> (p, s)	<i>Thalictrum dasycarpum</i> (b, r)
<i>P. serotina</i> (p, h, b, i)	<i>Tilia glabra</i> (h, B, i)
<i>P. virginiana</i> (P, B, s)	<i>Trifolium hybridum</i> (p, B, s)
<i>Ranunculus abortivus</i> (P, B, s)	<i>Trillium cernuum</i> (B, r)
<i>R. recurvatus</i> (p, B, s)	<i>Ulmus americana</i> (B, i)
<i>Rhamnus alnifolia</i> (B, r)	<i>Vagnera trifolia</i> (b, r)
<i>Rosa carolina</i> (b, s)	<i>Viburnum acerifolium</i> (P, H, s)
<i>Rubus canadensis</i> (p, B, s)	<i>V. cassinoides</i> (p, B, r)
<i>Rumex crispus</i> (elongatus) (p, s)	<i>Viola subvestita</i> (p, s)
<i>Salix lucida</i> (B, r)	

Relic species

Trees of the original forest tower over the aspens by 15-25 m., provided they have not been destroyed by fires (fig. 1). The most frequent are *Pinus resinosa* and *P. strobus*. From their seeds grow the trees which eliminate the aspens. Big tree relics are less likely to occur on the better soils because the aspen association seldom has a chance to develop immediately after lumbering. Should such an opportunity occur, the relics would be spindling trees rejected in the lumbering operations, in about 90 per cent of the cases either *Acer saccharum* or *Fagus grandifolia*, with occasionally *Tilia glabra*, *Betula lutea*, or *Fraxinus americana*. However, stump sprouts quickly shade out chance aspen seedlings. Repeated fires are more certain to burn up old trees in the beech-maple and bog forests than old pine trees.

The most numerous relics, however, are those "ground plants" whose underground parts survive the fires and send up new aerial parts. This is true of secondary species in most of the forest associations, but especially so of the pine association. For many species the particular kind of tree casting the shade makes little difference,

and such species appear to be normal to the aspen association. The other species disappear within two or three years.

A few especially prominent relics in the aspen association are *Aralia nudicaulis*, *Aster macrophyllus*, *Botrychium virginianum*, *Carex* (several species), *Chimaphila umbellata*, *Clintonia borealis*, *Cornus canadensis*, *Gaultheria procumbens*, *Pedicularis canadensis*, *Pinus resinosa* (large trees), *P. strobus* (large trees), *Trillium grandiflorum*, *Unifolium canadense*, *Vaccinium angustifolium*, *V. nigrum*, and *Vagnera racemosa*.

Invading species

Numerous invaders are present in the aspen association. They indicate the tree associations which replace the aspen association (4). The most frequent are pines in the sandy upland, *Acer saccharum* in the better uplands, and *Thuja*, *Abies*, and *Picea* in the wet ground. The number and exact kinds depend upon the abundance and proximity of seed-producing trees. Such invaders must be able to grow in the shade of the aspens. As this is usually less than in the virgin forest, the growth of invaders is more rapid in the aspen association unless the soil is too poor or too dry, or some other retarding feature prevents (1). The most important invaders are as follows:

IMPORTANT INVADING SPECIES

ON BETTER UPLAND SOILS.—*Acer saccharum****⁴, *Betula lutea**, *Fagus grandifolia****, *Fraxinus americana**, *Tilia glabra**, *Tsuga canadensis**.

ON POORER (SANDIER) UPLAND SOILS.—*Pinus resinosa***, *P. strobus****, *Quercus borealis***.

ON LOWLAND AND BOG SOILS.—*Abies balsamea***, *Fraxinus nigra***, *Picea glauca**, *Thuja occidentalis****, *Ulmus americana**.

Introduced species

The flora of the aspens is made up prevaillingly of native plants. For the most part the 25-30 introduced species are limited to the immediate vicinity of roads, and, as GLEASON and MCFARLAND (7), GLEASON (9, 11), and HELEN COBURN and DORIS DEAN (unpub-

⁴ *** Indicates a frequency index between 5 and 10; ** 1-5; and * less than 1.

lished paper) have shown, are closely dependent upon human activity for spread and even perpetuation. Only a few seem to be able to maintain themselves. The principal ones in point of number of individuals are, in order, *Poa pratensis*, *P. compressa*, *Rumex acetosella*, *Agrostis palustris*, *Phleum pratense*, *Trifolium pratense*, *T. repens*; but of these *Rumex acetosella* is unquestionably the most successful in the aspen association.

Fate of association

An aspen association developing on an area following one or two fires usually gives place to a higher genetic association in a comparatively short time. On the better soils this is the beech-maple forest within about 20-25 years. On wet ground there are two common possibilities. The *Thuja* association may enter immediately and replace the aspen association in 12-20 years, or a mixture of spruce-balsam attains ascendancy in an even shorter time. The lowland forest of *Fraxinus nigra* and *Ulmus americana* sometimes replaces it. In the absence of succession the aspen association continues and develops some medium-sized trees (16). On sandy upland it takes longer (usually at least 30-40 years) for the pine to displace the aspen association (fig. 12). Further fires favor continuance of the aspen association indefinitely. An intermediate stage of oak (*Quercus borealis*) obtains over several parts of the region, but does not add materially to the length of time for succession if pine seedlings are available. Fires as often as once in 12 years favor the aspens at the expense of the pines.

FIRES IN ASPEN ASSOCIATION (figs. 4, 6, 13).—If fire comes shortly after the aspens have germinated, it may be sufficiently severe to kill them outright and necessitate reseeding. In such cases the land may lie with little or no vegetation for two or more years. If the next fire does not occur until the aspens are well established, however, the underground parts are not killed and promptly regenerate. If fires happen at intervals of a few years, the aspen association is maintained. In a bog the chance of killing the underground parts is even less. Fires in boggy areas, while encouraging aspen perpetuity, also favor the development of willows, which sometimes maintain a temporary dominance in an area until replaced by bog associations,

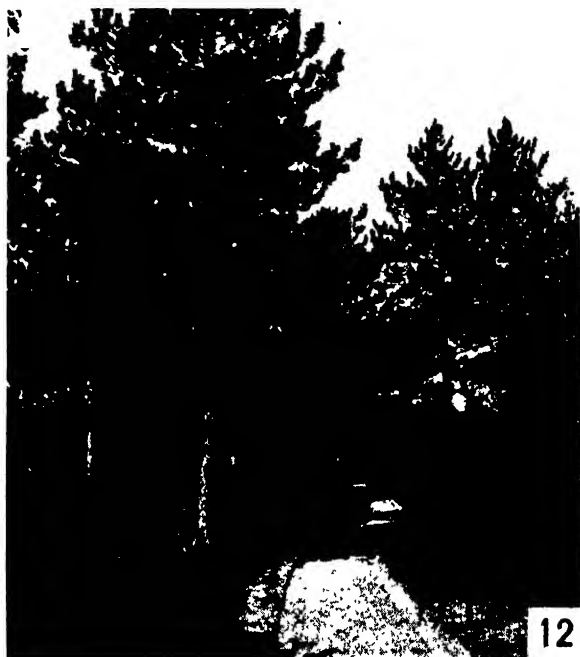


FIG. 12



FIG. 13

FIGS. 12, 13.—Fig. 12, view along Hogback Road showing complete ascendancy of pines over the aspens which ten years previously were dominant in this area (photograph by G. E. NICHOLS); fig. 13, bluegrass meadow resulting from yearly fires in aspen area east of Pellston; occasional clumps of *Diervilla diervilla*, *Rubus allegheniensis*, *Pteris aquilina*, and *Solidago hispida* visible; July 21, 1917.

or until they are again burned. In the better types of soil, if fires recur at short enough intervals, the aspen association will maintain itself indefinitely. If the fires are at long intervals the stump sprouts of beeches or maples compete for dominance and may attain it. If fires occur often, the aspens are favored, unless the fires are so frequent that even the aspens are killed, as is the case when fires occur annually. Under such conditions a meadow of *Poa pratensis* usually develops and maintains itself as long as such conditions continue (fig. 13).

In sandy land, fires even at rather long intervals (12-20 years) favor perpetuity of the aspen association. Fires at more frequent intervals favor a heath vegetation unless they occur as often as every other year, in which case either a meadow or open ground is the result. If the humus is actually burned out of the soil, then succession must start at the beginning. The seriousness of the situation in the case of sandy land lies in the fact that even an ordinary fire is rather certain to kill all small pine seedlings. Even a light fire is also likely to kill pine saplings under 1.5 m. high. As these pines do not sprout from their stumps, reseeding with all of its precariousness is necessary. One such area, near the Biological Station, favorably situated with regard to seed trees and other conditions, is now (1930) in its eighth year with but five pine seedlings in addition to the few left after the May, 1923 fire. The area had been noted for the large number of seedlings and saplings of pine.

In all cases burning opens up the ground to more light, which is followed by a great increase in the number of individual ground plants and usually also in number of species. Both diminish as shade becomes re-established. Table IV, arranged from class data by RUFUS MOORE, shows the relationship between data of last fire and frequency of coniferous and aspen trees. All of these sets were on sandy upland soil; that with least humus designated as "1."

MOSS AND LICHEN COVER (fig. 14).—In areas in which a moss and lichen cover is prominent, such as is often the case where fires have been frequent, the opportunity for ecesis is greatly diminished. The seedling starting on top of such a mat must force its way down through 5-10 cm. of dense moss or lichen growth, with only such water as is obtainable from the surface of the moss or lichen. Any

small rainfall or dew is absorbed by the moss or lichen, which can often hold it against the ability of a seedling to take it, so that the seedling dries before it has had opportunity to penetrate the moss cover (14). Favorable circumstances, such as continued cloudy, wet spells, are decidedly infrequent. One may find tree seedlings coiled up after a rainy spell, indicating their effort to force their way through such cover.

A small seed which has dropped through the cover to the surface may get its primary root into the ground, but with its cotyledons

TABLE IV
ABUNDANCE OF CONIFERS AND ASPENS AFTER FIRE

	ASPEN SETS					
	A	B	C	D	E	F
	Last fire					
	1925	1923	1909	Before 1909	1901	Before 1892
Percentage of all conifers. . .	0	0.2	1.3	4	11	34
Percentage of <i>Populus grandidentata</i> and <i>P. tremuloides</i>	95	61.0	55.0	69	44	35
Soil (scale 1-3)	1	3	3	2	3	3

enmeshed in the *Cladonia*, it may be pulled out by the changes in height accompanying soaking in rains or heavy dews (1). Such heaving was repeatedly noticed in 1926 (a dry summer) both naturally and experimentally, but was not noticed in ordinary (1927) or wet (1928) seasons. Not until the mat is broken by shade are seedlings successful (15), except as acorns buried by rodents may sprout in dense bunches.

RELIC ASPENS.—As the aspen dies when it is shaded, it is not long expected in succeeding associations. To remain as a relic the aspen tree must grow in height at least as fast as the tallest trees around it. In the best example in the region there are aspens as high as 20 m. and as thick as 35 cm., but balsam and spruce are now obviously gaining on them. In the sandy upland aspen trees cease to add materially to their height after 25-30 years, whether they are shaded or not. Trees 35 years old have badly discolored if not obviously

rotted wood. Death may be due to old age, windfall, heart rots, or less frequently to the work of borers.

The typical secondary species disappear as shade becomes denser, giving place to an increasing quantity of secondary species of the succeeding association, or by their disappearance aiding the openness of a succeeding association. In increasing shade, *Diervilla* and *Pteris* at first grow much larger, but as the shade becomes too dense or continuous they begin to die off. Establishment of a needle



FIG. 14.—Sandy upland aspen area in which many aspens have been removed by cutting, showing particularly the dense ground carpet of *Cladonia rangiferina* through which only a few plants of *Pteris aquilina* have appeared; August 5, 1926.

carpet on the ground eliminates *Pteris*, even though the shade may not be dense.

ASPENS IN RELATION TO CULTURE.—Aspens in this region occur almost entirely on uncultivated land. They offer no problems to the agriculturist or horticulturist except perhaps in the use of low wet land, but they will reclaim any abandoned land left free from cultivation, in the absence of too frequent fires.

Among the dominant species only *Betula papyrifera* is cultivated or planted for decorative purpose to any extent. Cultivation for pulpwood offers possibility of using otherwise idle land. ROBINOVÉ and HORTON (16) state that 30–35 years is about the right age for

harvesting on the lowlands where such culture is promising, and recommend the use of *Populus tremuloides* and *P. balsamifera*. *P. grandidentata* on sandy upland might be harvested every 20 years, but would yield much less.

Summary

1. The aspen association, a secondary one, is now the most extensively developed over the wide areas in the northern part of the lower peninsula of Michigan.

2. The association develops after the burning over of various associations. It occurs on each of the three prominent soil types. When on sandy upland soil it is dominated by *Populus grandidentata*, on clayey upland soil by *Prunus pennsylvanica*, and on lowland soils by *Populus tremuloides*.

3. The association is characterized by a large number of species.

4. Barring repeated fires, the association is likely to be replaced by the suitable forest association within 20-40 years, but with occasional fires it is perpetuated indefinitely.

5. Lowland conditions are most favorable to aspen exploitation in this region.

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CICATRIZATION OF FOLIAGE LEAVES

I. WOUND RESPONSES OF CERTAIN MESOPHYTIC LEAVES

ROBERT B. WYLIE

(WITH FOURTEEN FIGURES)

Introduction

The foliage leaf is necessarily the most exposed part of the plant, but at the same time functional limitations compel a structure which affords at best but partial protection. Primarily for photosynthesis, the leaf cannot employ massive or opaque coverings since these would prevent the entrance of light. In contrast to the extensive cork and bark of stems, foliage leaves have usually a single epidermal layer of which only the cuticle of the outer wall is really protective. While there are various modifications of structure, especially in plants of unusual habitats, high foliar efficiency involves the free exposure of a relatively delicate organ. The corollary to an autophytic terrestrial life is a plant body with chlorenchyma rather freely exposed and without adequate protection from dangers physical and biotic.

This enforced situation renders the foliage of plants peculiarly liable to injury. Winds, hail, insects, larvae, and grazing animals are destructive and their attacks leave the mutilated organs which are familiar to all. But the average leaf, while relatively helpless to prevent injury, seems peculiarly well fitted to care for its wounds. Even under severe attack by larvae the effects of injuries are localized and there is no marked wilting of remaining portions of injured leaves. Despite the frequency of lesion leaves seem to enjoy a remarkable immunity from traumatic infection. Remnants of foliage leaves are seen everywhere which continue to function for the remainder of the season without evidence of fungal invasion.

Such conditions suggest that under average conditions very efficient defenses are set up by the leaf immediately after injury, and that these operate both to lessen water loss from wounds and to inhibit the entrance of invading organisms. This paper is concerned

with the development of the protective barriers formed by certain mesophytic leaves following lesion. An attempt is made to trace these structures from their initiation to maturity and to outline something of their significance.

Literature

The wound responses of stems have quite naturally interested both practical and scientific workers. Similarly insect attacks leading to gall formation on leaves have been widely studied, as evidenced by the voluminous literature. The normal healing of foliar wounds, however, has received relatively less attention. Papers dealing with leaf cicatrization are few in number and there seems to be no connected account of cicatrice development. While manuals on plant pathology devote much space to leaf diseases, infections through foliar wounds have compelled relatively little attention. This situation indicates the efficiency with which the wounds of leaves in general are healed.

MASSART (7), in his general survey of cicatrization processes in the various groups of plants, devoted some attention to the leaf. He outlined certain results following wounding in a number of leaves, noted the death of cells along wounded margins, and figured tissue responses of *Nuphar luteum*, *Clivia miniata*, and *Hoya cavenosa*. Of special interest is his recognition of the difference in behavior of older and younger leaves of the same species, and also the contrast in response of wounded parts freely exposed to air in comparison with those partly protected from water loss.

BLACKMAN and MATTHAEI (2) noted that if leaves of *Prunus laurocerasus* var. *rotundifolia* were removed from the tree and kept in beakers with cut stalks in water they showed no healing reaction if cut with a sharp knife; but if tissues had been killed by any means which crushed a number of cells an abscission layer was subsequently developed, cutting out the dead tissue. Leaves if left on the tree developed layers of cork cells, after a preliminary browning of the edge, and with these there was no suggestion of excision.

WYNEKEN (15), in a paper unfortunately without illustrations, reported the results of his study of a considerable number of dicotyledons. He commented on the widespread development of wound cork

following leaf injury. The outside layer of the healing tissue had suberized walls, while in some cases there was lignification of the walls of cells next underneath the suberized layer.

BUSCALIONI and MUSCATELLO (3) carried out an extensive series of experiments studying the general reactions of leaves to various types of wounds produced by cutting, rubbing, crushing, and chemical destruction of cells. The healing tissues varied widely in leaves of different types, and in lesser degree with the nature and extent of the wound. They noted that humidity and light influenced wound responses and that detached leaves did not react strongly even when supplied with nutrient solutions. They observed a more active wound response in dicotyledons than in the leaves of monocotyledons and pteridophytes.

GERTZ (6), working with wounds caused by leaf miners, found commonly a marked hypertrophy of mesophyll cells bordering the tunnels. No periderm, however, was developed in any case along the cavities.

WOIT (10) reported on the general phenomena of foliar wound reactions, accompanied by a bibliography of important papers which in part are briefly reviewed. In this survey he found no data of special systematic significance. Simpler leaves developed simpler healing tissues while the thick leaved evergreens formed more massive wound cork. Where water loss was reduced, as along the tunnels made by leaf miners, the borders developed no wound cork and frequently showed but slight response. Thinner leaves formed a callus of modified wound cork which was more markedly developed in the thicker broad leaved evergreens. WOIT stressed the significance of atmospheric moisture, transpiration, and water loss from the wounded margins. He noted that in a moist atmosphere callus is formed, while a freely exposed wound develops wound cork only.

Numerous papers have dealt directly or indirectly with tissue responses of leaves, or other plant parts, following infection by fungi. No attempt is made here to review this literature but a few papers are noted.

DUGGAR (5) showed some time ago that the shot-hole effect on the foliage of plums, peaches, cherries, etc., is a peculiar reaction of the leaf to injuries by fungi, certain chemical agents, and possibly

other causes. He did not report unusual cellular development along the line of abscission. SAMUEL (9) studied the results of infection of almond leaves by *Claustrosporium*. In the majority of leaves the diseased portion was limited to a circular area which is usually cut out, producing the shot-hole effect and leaving the host free from infection. In young leaves the region of infection is invariably abscised. In older leaves, especially in late summer or autumn, the diseased part is retained in place. Cell divisions lead to the formation of wound cork, however, which checks further progress of the fungus.

BARTHOLOMEW (1), in a study of the *Alternaria* rot of lemons, noted that there is formed a resistant layer of corklike tissue somewhat in advance of the invading mycelium. In some cases two or three successive barriers had been formed at intervals by the host to check the invading growth. His figures show a cork zone of considerable thickness. CUNNINGHAM (4) found a number of leaf diseases resulting in a definite cicatrice about the margin of the diseased area. Artificial foliar wounding resulted in some cases in the formation of a cicatrice similar to that formed in diseased leaves. In other cases the diseased leaves showed no evidence of a cicatrice such as is formed by the wounded leaves of these species. In two forms he reported that neither wounded nor diseased leaves developed a specialized cicatrice.

Some time ago the writer carried out some simple experiments (11) which illustrate the marked capacity of foliage organs to withstand injury. Leaves of certain woody plants may live with many or even all of their major veins transected. Using a leaf-punch most of the blade may be cut away, while remaining portions continue to function even though the total of freshly exposed wounded margins is many times the length of the blade. By means of variously oriented excisions the flow of materials in the minor venation may be forced in any direction and their carrying capacity shown to be enormously greater than that demanded under normal conditions. Another paper (12) outlined certain results of a preliminary study of the healing tissues developed following foliar lesion. It was noted that the cicatrice proper is derived mainly through new cells resulting from mitoses involving all cell layers of the blade. The width of

the cicatrice varies considerably, but seems to bear a rather definite ratio to the thickness of the leaf itself; the thicker the leaf the wider the zone of scar tissue developed after lesion. An exception should be noted in the case of succulent or semisucculent leaves. A constant factor also is the presence of suberin and lignin in the cicatrice region.

In a later paper the writer (13) summarized further facts relative to the cicatrice proper, and called attention to the preliminary barrier, termed by him the *pseudocicatrice*. This initial barricade results from the death and collapse of mesophyll cells following wounding, and protects the exposed margin. The shrinkage of these dead cells usually pulls inward one or both epidermal layers with their highly protective cuticle. Secretions such as latex may also be liberated along the wounded margin which would further increase the efficiency of this barrier. The pseudocicatrice is formed quickly following lesion, checks traumatic water loss, and soon ends the death of tissue adjacent to the wound. It is probably an important factor also in lessening the danger of infection following foliar lesion. An additional paper (14) traced the development of both pseudocicatrice and cicatrice in leaves of *Citrus limonia*. Leaf structure and wound responses of leaves grown in an Iowa planthouse were compared with those developed in a California orchard. It was noted that barrier tissues are of much slower development than in mesophytic leaves, and are much more massive when mature. The cicatrice of the lemon leaf requires two or three weeks for its development.

Methods and material

After a general survey involving a considerable number of deciduous plants, *Vitis vulpina* and *Rhus glabra* were chosen for special study. Both are woody angiosperms which develop new foliage during the greater part of the summer, thus making available leaves of varying ages at any time of the growing season. In both the leaves are strongly dorsiventral in organization. The leaves of *R. glabra* have a more highly specialized structure and milky juice, while those of *V. vulpina* have simpler leaves which are devoid of latex. Of the two *R. glabra* seems more xerophytic, although the actual distribution of plants seems scarcely to bear out this suggestion. In comparison with these forms some study was made also of *Syringa vulgaris* and certain data relative to this species are included.

Since earlier studies had demonstrated that there is considerable difference in the traumatic response of immature as compared with leaves fully formed, two series of experiments were carried out, one with younger and the other with well developed leaves. Since observations on the wound responses of these species showed that for fully exposed leaves the nature of the cutting tool did not materially influence the cicatrice, the wounds most closely followed in this study were made by means of scissors. It seemed that the crushing effect of the blades would more closely approximate the type of injury caused by insect mandibles. A parallel series wounded with a sharp razor was collected for comparative study.

The leaves were cut, usually in the outer portion of the blade, at right angles to the major veins. In all experiments leaves were wounded early in the forenoon of clear midsummer days, thus exposing the freshly cut margins during the hours of greater transpiration and under conditions that favored traumatic water loss. Material was collected on alternate hours during the first day, then daily for ten days, and at five-day intervals up to about thirty days. At indicated intervals marked leaves were taken from plants and strips were removed by cuts parallel to the wounded edges. These excised portions were then divided into convenient rectangles, fixed in chromacetic acid, and sections cut uniformly at $12\ \mu$ thickness. Most experiments were carried out and considerable material was collected on the grounds of the Iowa Lakeside Laboratory by Lake Okoboji in northwestern Iowa. Further material for all three species was also collected at Iowa City, in east central Iowa.

1. *Vitis vulpina*

All the leaves used as the basis for drawings in this study were sun leaves on a wild grapevine growing along the exposed south side of a residence building in Iowa City. While some leaves were subsequently shaded by overgrowth of adjacent branches, only those in full sunlight at the time of wounding were used. As noted, two series were run, mature sun leaves and also partly developed leaves which were 1-3 cm. long at the time of wounding.

The leaves of *V. vulpina* range in thickness from 120 to 175 μ , with an average cross dimension of about 150 μ . The simple epidermal layers (figs. 1-4) are nearly equal, with an average thickness of about

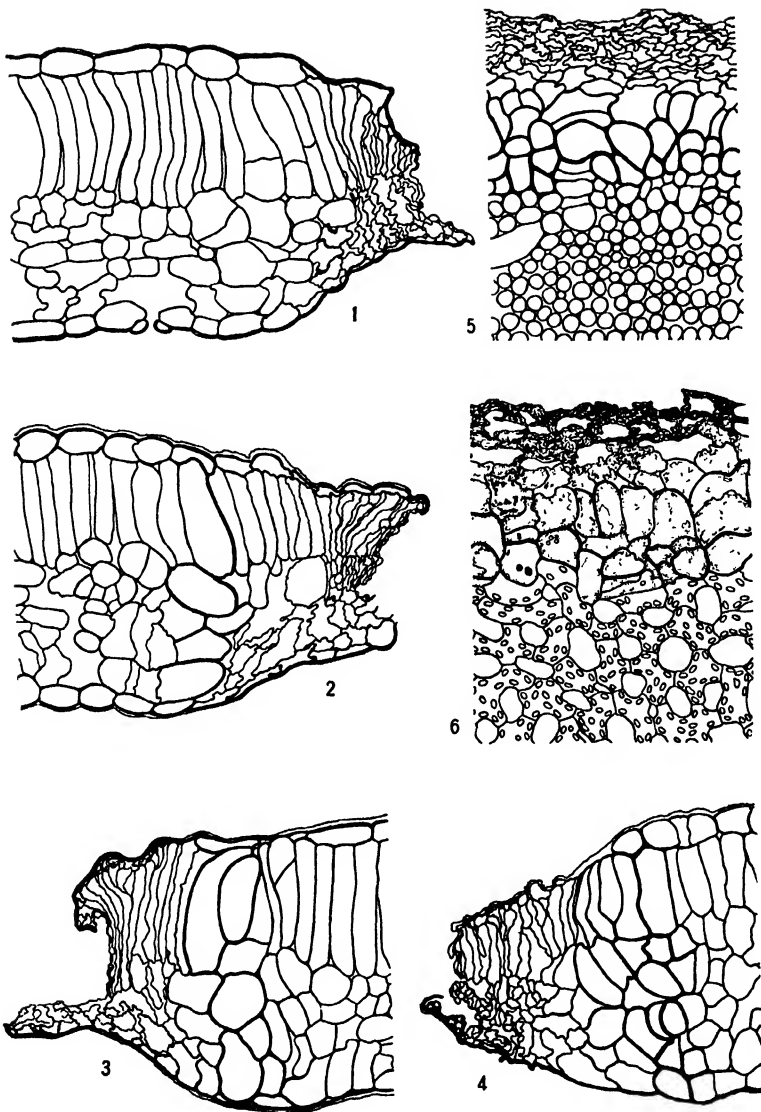
14 μ , but the upper has a much heavier cuticle while the lower bears most of the stomata. The palisade extends for 50–60 μ and is usually a single layer. The spongy mesophyll, 60 μ in average thickness, appears disconnected and irregular in the transverse aspect but shows a beautiful meshed arrangement in sections parallel to the leaf surface (fig. 6). Only when viewed in this direction is the unity of this layer and the real organization of the leaf apparent. The cells of the spongy mesophyll layer are drawn out horizontally in lobes which join about air spaces in a symmetrical way. Large openings thus extend from the stomata through this layer and into the palisade, where lesser spaces surround the elongated cells (figs. 5, 6).

While the major veins involve the entire leaf thickness, or protrude as ridges on the under side, the minor venation lies imbedded in the upper portion of the spongy mesophyll. By ramifications of these smaller veins all parts of the chlorenchyma are brought into close connection with conductive channels. Measurements in the plane of the islet borders reveal an average intervascular distance of 200 μ for this species; the average maximum distance to a vein is therefore but 100 μ , and most of the chlorenchyma is inside this limit. Tissues higher or lower are proportionately removed, but these border on the epidermal layers which assist both the functions of conduction and mechanical support.

PSEUDOCICATRICE

A zone of tissue of varying width is crushed in the wounding process. Water escaping from the unprotected margin soon results in the death and collapse of additional cells in the tissue running parallel to the wounded edge. In *Vitis* this strip is 15–50 or more cells in width following wounding with scissors. If cuts are made with a sharp razor a somewhat wider zone of tissue dies, due no doubt to the greater exposure of intercellular spaces. While the razor crushes fewer cells, the air spaces are left more open to water evaporation and the total loss is thereby usually increased. Wound responses in other respects seem to show no difference.

Sections cut parallel to the epidermis show that the line of junction between living and dead tissue extends directly across islets and minor venation but usually dips inward where larger veins are cut across by the wound. One reason for this may be the greater loss



FIGS. 1-6.*—*Vitis vulpina*: fig. 1, cross-section of leaf 4 days after wounding, showing pseudocicatrices and initial cell divisions of cicatrice formation; fig. 2, 8-day stage with differentiation of outermost layer of cicatrice underneath pseudocicatrices; fig. 3, 12-day stage, showing nearly mature cicatrice; fig. 4, 23-day stage in cross-section, showing typical pseudocicatrices and cicatrice; fig. 5, 23-day stage, palisade region as seen in section parallel with epidermis; fig. 6, from same slide as fig. 5, but showing section through spongy mesophyll.

* All figures are magnified approximately 305 diameters. Figs. 1-11 were made by projection, using a Carl Zeiss no. 20, 8.3 mm. apochromatic objective in combination with a Zeiss 15X compensating eyepiece. Figs. 12-14 are photomicrographs used as foundations for drawings; negatives were made with same objective in combination with a no. 1 Homal.

of water from interrupted tracheal tissue, but another important factor is the difficulty of developing a pseudocicatrice with the epidermal layers held out on both sides by the thickness of the adjacent vein.

Transverse sections of the leaves (figs. 1-4) show the relations of collapsed mesophyll, epidermal layers, and living tissues within as the pseudocicatrice develops. The dead mesophyll cells shrink, their walls collapse into folds, and the epidermal layers are usually pulled inward or may even overlap on the wounded margin. Lignin usually appears in the pseudocicatrice on the third or fourth day, and at about the same time it may be demonstrated in the cicatrice region underneath.

In *Vitis* the pseudocicatrice is obvious on exposed leaves within an hour after wounding, and seems to change little after the first day. Its development tends to check traumatic water loss and soon stops the dying back, as there seems to be no marked shift of the line of demarcation between living and dead tissues after the first few hours. Even in seriously injured leaves the areas of tissue that later die are well marked within the first day, and were probably determined by the water losses immediately after wounding while the pseudocicatrice was forming.

CICATRICE

There are no marked changes in the marginal living cells underneath the pseudocicatrice for a couple of days after wounding. On the third day, as noted in the series most carefully followed, cell enlargement begins in a zone extending through the leaf parallel to the wounded margin. Increase in size of the cells of this general region rapidly diminishes the intercellular spaces, which are soon occluded by the encroachment of these enlarging cells. The outer living cells become convex, since the adjacent tissues have collapsed and no longer offer support. The cicatrice as a whole, in this species and in others, tends to be convex on the outer side and in some is strongly outcurved.

The fourth day showed cell divisions in all cell layers (fig. 1), including both upper and lower epidermis. The new walls laid down in epidermal cells and in the spongy mesophyll are approximately parallel with the wounded margin. The long palisade cells usually

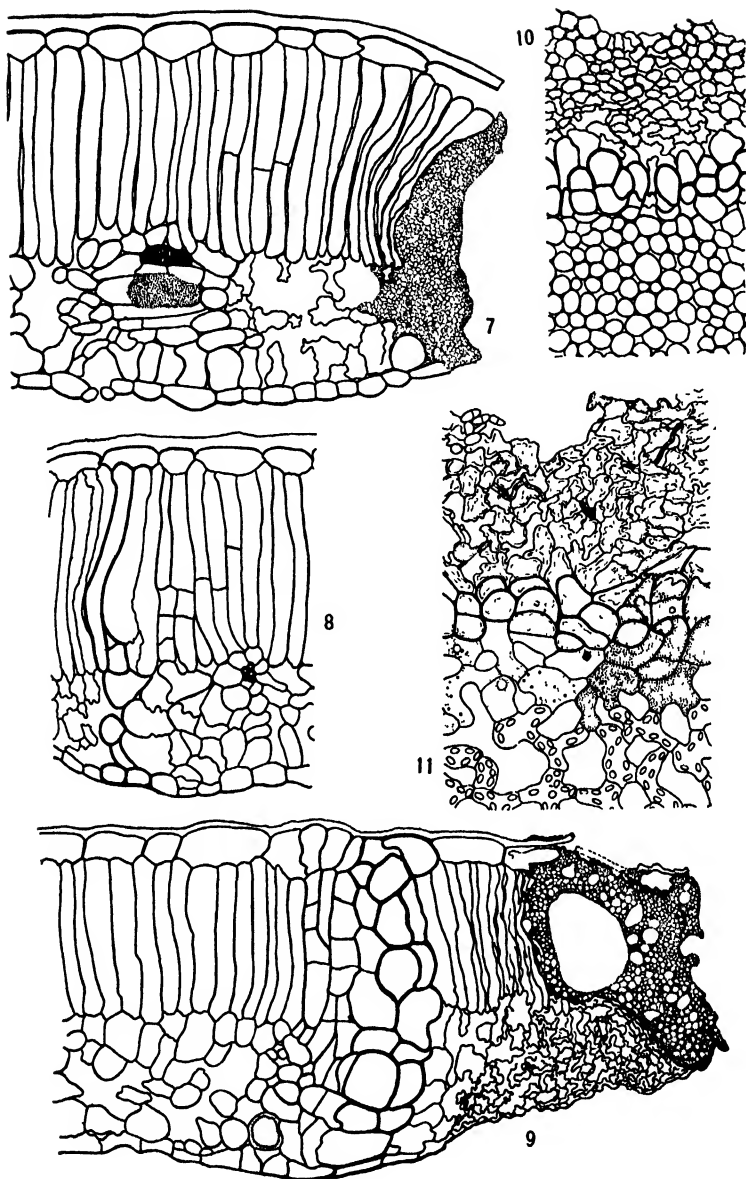
divide first into two or more shorter cells and these may later divide vertically. The cells of the palisade that enter into the outer part of the suberized zone of the cicatrice often remain undivided, however, the inner ones breaking up more freely. During the third and fourth days lignin is deposited in this general region of activity, and may generally be demonstrated also in the pseudocicatrice. Suberin is usually somewhat later in appearance in this species, but may be demonstrated in the developing cicatrice by the seventh day and becomes increasingly abundant as it matures. By the eighth day all mitoses necessary for the cicatrice have been completed and the cells are undergoing marked modification (fig. 2). Unstained sections at this time plainly show the yellowing of walls and changes in the content of these outer cells. The walls thicken considerably, the secondary lamellae showing clearly; but such thickenings are generally omitted along the interior faces of the innermost layers of the cicatrice next to the living tissue. By the twelfth day the healing tissue of *Vitis vulpina* has taken its mature expression. Subsequent collections at intervals up to 30 days showed little change, and differences noted were probably due to the individual behavior of various leaves and to modifications due to proximity of veins.

In most leaves there is a series of compact, flattened, and living cells immediately beneath the cicatrice. These persist without enlargement or specialization and retain their cambium-like appearance. This zone is much more pronounced in the thicker broad leaved evergreens (figs. 4-6, 13).

The mature cicatrice of *Vitis vulpina* averages five or six cells in width, and, as noted, develops primarily from new cells resulting from cell division in all layers (figs. 3-6, 13). Its structure is uniform in organization away from vascular strands, but near veins it is usually considerably modified, often much thicker, and frequently more irregular. Veins are obstacles rather than aids to cicatrization. The main defensive layers have thickened walls which are suberized and constitute a most efficient barrier in every way (fig. 13).

2. *Rhus glabra*

The leaf of *Rhus glabra* presents a strongly specialized dorsiventrality with well marked xerophytic tendencies (figs. 7, 12). The leaves studied varied in thickness from 160 to 200 μ , with an average.



FIGS. 7-11.—*Rhus glabra*: fig. 7, transverse section of leaf 4 days after wounding; latex (stippled) in pseudocicatrice; fig. 8, 6-day stage, showing cicatrice region (pseudocicatrice not shown); fig. 9, 25-day stage, showing normal leaf tissue, cicatrice, and pseudocicatrice; fig. 10, 25-day stage, from section through palisade cut parallel with epidermis; fig. 11, from same slide as fig. 10, but showing section through spongy mesophyll.

distribution as follows: upper epidermis $21\ \mu$, palisade $100\ \mu$, spongy mesophyll $50\ \mu$, and lower epidermis $10\ \mu$. The upper epidermis is covered by a thick cuticle, giving to the leaf its glossy appearance. All the palisade cells are long and slender, making up nearly three-fifths of the mesophyll, and these usually remain undivided in the normal leaf. The stomata are all in the lower epidermis, which is much thinner than the upper layer and has a relatively delicate cuticle. Leaves used were fully exposed sun leaves, collected in one series on the grounds of the Lakeside Laboratory in northwestern Iowa, and also from plants in Iowa City.

Sections cut parallel with the epidermis revealed the spongy mesophyll, as in *Vitis*, with cells in meshlike arrangement (fig. 11). This structure is achieved by lobings of the cells, some having several extensions, each meeting a corresponding lobe from a neighboring cell. This symmetrical arrangement is scarcely suggested in the transverse section and probably results from lateral tensions developed during formation of the leaf.

The palisade layer, in sections cut parallel with the epidermis, is revealed as a series of rounded columns (fig. 10), surrounded laterally by air spaces, except at their upper ends where they make contacts with each other near the plane of their junction with the epidermis. Even in this region there may be small intercellular spaces where the cells are pulled slightly apart at the corners. The large upper epidermal cells each have 6–10 palisade cells attached on the under side.

Vascular tissue other than the major venation lies imbedded in the spongy mesophyll. The ultimate branches of the veins lie unusually close together in this species. Measurements from microtome sections cut parallel to the epidermis showed an average intervascular interval of only $85\ \mu$. Most of the smaller conductive strands are not visible when the whole leaf is examined. There may be a correlation between the extreme length of palisade cells and the close proximity of vascular strands which are closely associated with the lower ends of these cells.

Rhus possesses a vigorous latex system which must be taken into account in studying its reactions to wounds (figs. 7, 9, 12). Latex tubes are associated with all of the larger veins, and these pour out

a profuse discharge whenever interrupted. If an exposed leaf is cut across, leaving a smooth wounded margin, however, the milky juice does not always cover the edge completely but may dry in masses about the ends of the transected veins. On the other hand, if a freshly wounded leaf is placed under a bell jar the exuded latex soon covers the entire margin. Similarly if wounded in the evening or at night, the milky juice spreads out considerably more than when in the sun and exposed to air currents.

PSEUDOCICATRICE

The pseudocicatrice formed by *Rhus glabra* is quite different from that described for *Vitis*. There is usually a wider zone of dead tissue, and the latex enters prominently into the region of crushed cells. Often, however, the zone of dead tissue is wider under the dried latex masses than elsewhere. In addition to modifications associated with latex, the heavy upper cuticle and the enormously elongated palisade cells introduce modifying factors. The upper epidermis, because of the heavy cuticle, stands out more stiffly and curves less sharply over the wounded margin (fig. 12), but the thinner lower epidermal layer is more readily pulled in with collapse of mesophyll cells (figs. 9, 12). In the interior of islets, away from obstructing veins, the epidermal infoldings are well developed except where the latex has involved the margin. In all cases the epidermal cells, although collapsed, still have the heavy cuticle which exercises its protective function. Since there are no mitoses until the fourth day, and the permanent cicatrice requires about two weeks for complete development, it is obvious that the pseudocicatrice is the only defensive barrier for some time.

CICATRICE

Sections of leaves collected at intervals through two days after wounding showed no growth responses. It is difficult even to distinguish between living and dead cells along the border for some time; since the zone of dead tissue is usually wide the inner cells collapse rather slowly. About the third day, however, differentiation is evidenced by enlargement of the outer living cells. Mitoses begin in the palisade layer on the fourth day, when the long palisade cells divide transversely into shorter units (fig. 7). Division is more active

on the fifth day, and by the sixth a single original palisade cell may have given rise to as many as four or more cells (fig. 8). In the spongy mesophyll cell division is somewhat delayed. Enlargement proceeds until cells make contact, then mitoses begin with new walls laid down parallel to the wounded margin. By the sixth day there is a compact zone of tissue in the region of the cicatrice; divisions meanwhile have taken place in both epidermal layers covering this expanding cicatrice region. Four, five, or more layers of the living cells enter actively into cicatrice formation from the sixth day forward. By that time some cells that have enlarged considerably present double the width of normal palisade. Yellowing of the walls, as shown in unstained sections, appears at this stage and is indicative of suberization. From the fourth day forward the cicatrice region reacts positively to tests for both lignin and suberin.

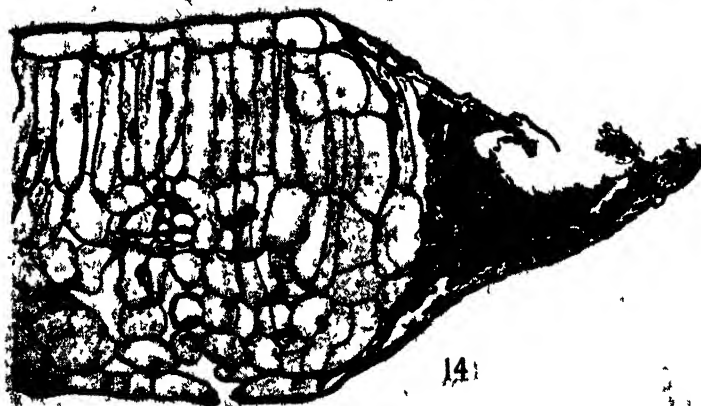
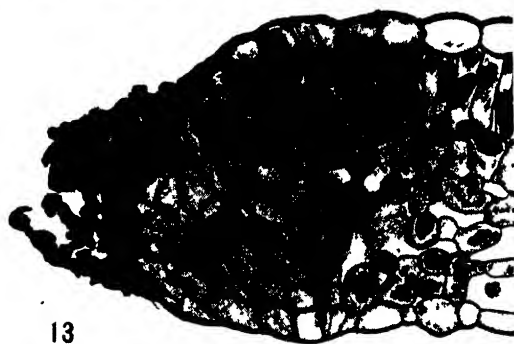
Changes in the cicatrice continue for some time, and approximately two weeks are necessary for its development. Modifications include enlargement of cells, thickening of walls, changes in cell content, and lignosubерization of cell walls throughout the barrier tissue (figs. 9-11). While the completed cicatrice proper includes but four to six rows of cells, there are usually several additional layers of relatively unspecialized cells compactly arranged on the interior of the cicatrice.

The mature cicatrice as seen in stained section consists of a compact tissue of highly modified cells (arranged usually in convex form) and involving all cell layers, including both upper and lower epidermis (figs. 9-12). Usually one or two cell layers have different structure, staining brilliantly with Flemming's triple stain and displaying a strikingly different content.

3. *Syringa vulgaris*

The leaf of *Syringa* is somewhat thicker than the preceding two, averaging in the material studied about 180 μ in this dimension. Its general structure (fig. 14) seems to be intermediate compared with *Vitis vulpina* and *Rhus glabra*; the average intervascular interval of 103 μ is also between measurements for the other species studied.

Events following wounding may be summarized briefly by com-



FIGS. 12-14 —Fig 12, transverse section of *Rhus glabra* showing normal leaf tissue, cicatrice, and pseudocicatrice; fig 13, transverse section of *Vitis vulpina* showing normal leaf tissue, cicatrice, and pseudocicatrice; fig. 14, transverse section of *Syringa vulgaris* showing normal leaf tissue, cicatrice, and pseudocicatrice.

parisons with the preceding species. Owing to the softness of the *Syringa* leaf and thinness of its cuticle, the pseudocicatrice forms quickly. With the death of exposed tissue the wounded margin is covered by the collapsed mesophyll, and the epidermal layers are drawn inward (fig. 14) except where blocked by adjacent veins. The cicatrice is of simpler type, consisting of two or three layers of specialized cells, and is strongly outcurved (fig. 14). The suberized walls of the outer layers are about the same thickness as in the other species studied, 0.8–1.5 μ , averaging nearly twice that of unmodified mesophyll cell walls. The cicatrice region responds positively to tests for both suberin and lignin after the sixth day following wounding.

4. Immature leaves

Wound responses of immature leaves were very different from those of older ones on the same plant. Wounded when the blade is but 10–15 mm. long, the softer tissues and less specialized epidermal layers readily collapse and permit an efficient pseudocicatrice. The epidermal layers are commonly drawn more abruptly inward, and often flatten out parallel over the dead tissue. The resulting pseudocicatrice, while no more extensive, is of a somewhat different type from that produced by older leaves under the same conditions. There is a greater degree of shrinkage of marginal mesophyll, resulting in a very compact pseudocicatrice. The cicatrice likewise shows general differences. It is more restricted and there is less cell enlargement. The protective cells are more nearly isodiametric than in older leaves of the species. Further experiments are in progress which deal with the varied responses of leaves when wounded at different stages of their development.

Discussion

The foliage leaf as the primary vegetative organ of the plant presents a series of correlated specializations the refinements of which are often hidden by their seeming simplicity. Its general structure, relative size, protective coverings, and internal organization result from compromises among numerous and sometimes conflicting factors. No less specialized are the foliar responses to wounds

which become inevitable when such organs are freely exposed in aerial position. Mesophytic leaves in particular respond promptly when injured.

The pseudocicatrice in its varied expressions is the initial barrier of the wounded leaf and for some time constitutes its only protection. It seems to be uniformly developed by exposed mesophytic foliage and is also formed by broad leaved evergreens, so far as studied by the writer, with the exception of the mature leaves of *Berberis nervosa*. Its failure to develop, owing to excessive humidity or to unusual rigidity of the walls of mesophyll cells, leaves wounds open to traumatic infections and retards or inhibits cicatrice formation.

Since the pseudocicatrice results primarily from the death and collapse of normal leaf tissue, there is no delay in its formation such as is necessary for the development of new cells or the gradual modification of living tissues. Under usual conditions both the need for protection and the development of the defensive barrier are related to the common factor of traumatic water loss. Obvious within a few minutes after wounding, the pseudocicatrice of mesophytic leaves soon lessens water loss and usually stops the further death of tissue within a few hours following lesion. Its efficiency is greatly increased by the cuticular layers which usually are drawn inward by collapse of mesophyll and epidermal cells.

The cicatrice proper, while highly efficient in its completed form, is relatively slow in its development. It is not initiated until three or four days after wounding, and its formation is related to the preliminary barrier. For several days after injury the marginal living cells, covered and protected by the pseudocicatrice, are passing through the cell division and cell enlargement stages. Ten or more days may be required for completion of the cicatrice in mesophytic leaves, and 20-30 days in broad leaved evergreens.

During the formative phases of the cicatrice it affords little protection to subjacent parts; indeed the foliar phellogen is itself protected by the overlying pseudocicatrice through this period. Subsequently the true cicatrice takes on the general aspects of cork through thickening and suberization of its cell walls. In the mesophytic leaves studied, suberin is seldom demonstrable before the fifth day and may be even later in its appearance. Lignin is usually pres-

ent in the cicatrice region and is sometimes found in the pseudocicatrice as well.

It seems probable that development of wound cork is in considerable measure contingent upon the prior development of the pseudocicatrice. PRIESTLEY (8) calls attention to the necessity for a "block" before there can be the formation of phellogen under the surfaces of certain plant wounds. When conditions inhibit formation of a pseudocicatrice, as noted by BLACKMAN and MATTHAEI (2) in the leaves of *Prunus* kept in moist chamber, or WOIT'S (10) observation of cells along the tunnels of leaf miners, there is likewise no cicatrice development. Under such circumstances the wound reponse, if any, is merely an enlargement of exposed cells with or without suberization of outer walls. A paper by the writer, to be published soon, deals with wound responses of *Berberis nervosa*. In this species the mesophyll cells of older leaves have walls so thick that they do not collapse when the protoplasts are killed, thus preventing the formation of a pseudocicatrice and likewise inhibiting the development of wound cork.

It seems probable that the ability to deal promptly with their wounds has been an important factor in the evolution of foliar organs. There is doubtless a direct correlation between the general type of leaf structure, with its corresponding liability to injury, and the capacity for prompt development of buffer tissues when wounded. The relatively thin and soft but highly efficient mesophytic leaf, subjected to constant injury, is possible only because its structure favors ready response to wounds and particularly prompt development of the pseudocicatrice. Broad leaved evergreens with better protected foliage are much slower in their wound responses. A survey of gymnosperms would probably show that their mature needle leaves have but limited capacity for the development of healing tissues. The mesophytic leaf, which from its organization is peculiarly liable to injury, is through the same structural qualities admirably fitted to deal with its lesions.

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SEX REVERSAL AND THE EXPERIMENTAL PRODUCTION OF NEUTRAL TASSELS IN *ZEA MAYS*¹

JOHN H. SCHAFFNER

(WITH FOUR FIGURES)

Introduction

The fact of sex reversal has been established for many species of plants. Commonly the reversal is brought about as readily in one direction as the other, namely, from female to male and from male to female condition. When such reversal takes place the physiological state must necessarily pass through a zero or neutral point in the transition from one sex to the other. The characters developed in tissues which have passed to the neutral condition are often of great interest; and when the general nature of the species is known, together with its comparative place in the evolutionary series, it usually becomes possible to predict with more or less certainty what is to be expected under a given environment. The writer has at various times made such predictions, usually with unqualified success. Thus in primitive monoecious or dioecious species, bisporangiate flowers are frequently produced on the neutral transition zone, while in those species which have highly evolved and reduced flowers complex sex mosaics may appear, even extending to the individual floral parts. In other cases rudimentary flowers or peculiar vegetative structures are produced.

Having discovered a method of controlling the sexual states in the tassel of *Zea mays*,² so that a given plot can be developed with approximately any desired percentage of individuals showing tassels with a greater or less degree of femaleness, even up to 100 per cent, and having observed that the neutral condition causes the development of an extremely vestigial type of tassel and spikelets, it became evident that ecological conditions might be so manipulated that a

¹ Papers from the Department of Botany, The Ohio State University, no. 249.

² SCHAFFNER, JOHN H., Control of sex reversal in the tassel of Indian corn. BOT. GAZ. 84:440-449. 1927.

considerable percentage of individuals would develop with entirely neutral, vestigial tassels.

Zea mays has evolved to an extreme and complex hereditary constitution, attaining evolutionary limits along many lines. The functional gradients developed in its ontogeny must be understood in order to interpret the peculiar conditions which changing environments bring about and to prepare for desired results. Corn is an annual with a definitely determinate terminal bud which ends its growth and life with the reproductive process. This fact is mainly responsible for the remarkable vestigial tassels produced experimentally. The latter part of the growth of the main axis, therefore, goes through a definite gradient leading to determination and death. The main vegetative axis also passes from a neutral condition to a secondary male condition during the latter part of its development, so that when conditions become proper for reproductive activity this male state gives a definite male dimorphism to the involved structures. The stem and leaf sheath become slender; the carpellate parts of the flower remain vestigial; the branching inflorescence factors are thrown into activity; the glumes show prominent secondary sex limited characters; and of course the stamens develop as normal structures. There are also definite physiological gradients, as increase in pH and increase in catalase activity. The gradient of differentiation in the inflorescence does not follow cell lineage, but the spikelets develop first somewhat above the middle of the main axis and also at about corresponding points on the branches. From this middle point development proceeds upward to the determinate tips, and at the same time downward to the base of the inflorescence. These movements are of great importance and are responsible for different modes of character expression with increasing or decreasing photoperiodicity.

A long light period of 13-15 hours' daylight produces a completely staminate tassel, other things being proper, but a daylight period of 8-10 hours causes a decided movement toward femaleness, so that in extreme cases very good ears are developed, and 100 per cent of the individuals will show at least some silks with normal ovularies in the tassel (fig. 1). The short illumination period, with the light coming through the greenhouse glass, with normal growing temperatures,

and with abundance of water and well manured soil, changes the functional gradient of the main stem so that instead of rising to the level where the sex balance swings to maleness it is lowered and passes through the neutral condition to the level of femaleness. It is evident, therefore, that if the neutral point is reached in the system just when reproduction and determination are beginning, it will be impossible for the hereditary factors, for spikelets, flowers, and other characters to come properly into play, since there is neither male nor female condition present in the cells. The result is a mere vestige of the inflorescence (figs. 1, 2). If the physiological condition has been changed more extremely, the female state will be present in the cells, normal female reproductive processes will be initiated, and normal female structures expressed (figs. 1, 3).

DEVELOPMENT OF SEX MOSAICS

Since the various gradients naturally do not all follow the same sequence as the cell lineage, complicated expressions will often result. On a decreasing light schedule at the proper balance, the change of gradients from maleness to femaleness will not have been attained when the differentiation of spikelets begins at the middle of the inflorescence axis perhaps, but may be only near this point. In such a case normal staminate spikelets will develop at first, but as differentiation advances toward the tip and the base the functional change will be developed to the neutral point, and the upper and lower ends of the inflorescence will be vestigial and neutral (fig. 2). Thus sex mosaics of varying degrees of normal staminate tissue in the middle and neutral tissues at the two extremes are abundantly produced. But if the movement is a little more prompt and the neutral point is reached and passed before completion of growth of the extremes of the tassel, then the physiological state will throw the sex balance to femaleness below and a sex mosaic of four zones will be produced, female at the base, next neuter, then male, and finally a neuter tip (fig. 3). The neutral zone below may often be practically absent, of course, depending on the rapidity with which the change from maleness to femaleness takes place in the differentiating system. There will be no femaleness at the tip because of the rapid developing of the determinate gradient, which will not give time nor condition for the



FIG. 1.—Three “tassels” from plants of same plot, developed in decreasing short light condition: (1) normal (male), (2) vestigial (neuter), (3) carpellate (female with neuter tip).

turnover from neutral to femaleness before determination of the axis is accomplished.

Thus also with autumn conditions of decreasing photoperiodicity, there will usually be not more than half an ear developed, for the system will be at zero when flower differentiation begins. Then the gradient downward will fall to femaleness and continue so to the base of the inflorescence; but the gradient upward will stay at zero be-

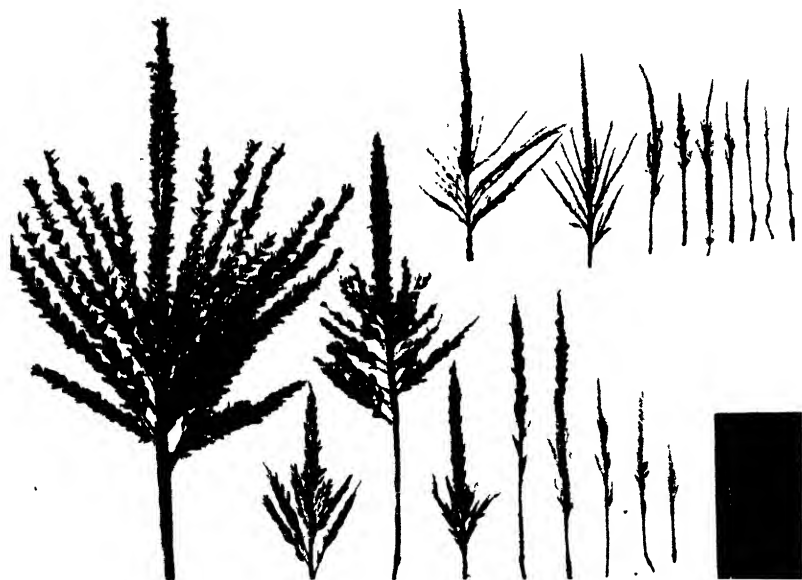


FIG. 2.—Series of tassels from normal (at left) to extreme neutrality (at right); intermediate tassels have vestigial tips or completely vestigial branches.

cause a determinate condition is rapidly developing and because adverse conditions of nutrition and growth are daily becoming worse. Under decreasing autumn light the tips have always been neuter vestiges, no matter how perfectly femaleness was developed at the base.

On the other hand, if the corn is developing in a suitable daylight period and this is lengthening as growth proceeds, the functional changes in the system below, or the substances accumulating, may act on the sex balance of the inflorescence and the differentiation gradients in such a way that not only will the system be thrown to

femaleness below, with normal maleness in the middle, but femaleness may also have an opportunity to develop at the outer ends, in rare cases even at the very tips of the main axis and branches. Frequently the mosaic produced will be complicated and tassels will be formed with isolated carpellate spikelets scattered about. Such tassels when developed to maturity have a striking appearance, the mature isolated grains standing out like pearls among the dry staminate



FIG. 3.—Series of five sex mosaic tassels at left, neutral tassel in middle, and four carpellate tassels with neuter tips at right.

nate spikelets. The specific production of these sex mosaics of various types in the tassel is due entirely to the nature of the environmental conditions and not to diverse combinations of genes of male, neuter, and female determining value. The hereditary balance of genes in the system has nothing to do directly with the particular mosaic produced. This is dependent on the development of the various physiological gradients induced, which in turn are dependent on the specific type of environment. Of the various environments, each produces a distinct type of inflorescence, whether completely male, completely female, completely neuter, or any of the diverse sex

mosaics, from the same balance of potentialities. Any theory of sex which would explain the diversity of sexual expressions by an appeal to a diversity of gene constitutions is entirely beside the mark. Such conceptions and hypotheses are fundamentally incorrect because they do not agree with established facts but merely with assumptions derived from facts which are amenable to entirely different and consistent interpretations.

There seems to be some confusion in regard to sex factors, conceived of as male, female, and neuter determiners and factors or genes responsible for specific male, female, and neuter characters. There is no dispute about the latter kind of genes and characters dependent on them. These apparently are present in great numbers and diversity in both plants and animals, as are other types of potentialities which are at the base of the various vegetative systems. Such potentialities follow the regular Mendelian program, but there is no direct evidence for postulating Mendelian factors for maleness, femaleness, and neutrality.

We have advanced far enough at present to be able to take a single grain of corn and make a somewhat safe prediction as to whether it will develop a pure staminate tassel or a decidedly carpellate inflorescence. We can also predict, in a percentage probability, whether the given grain is to develop a neutral tassel or a sex mosaic of one of the four general possible types, a neuter-male complex, a neuter-female complex, a male-female complex, or a female-male-neuter complex. The specific determination of the result is dependent on the environmental balance and not on a change of balance of genes, since no change of hereditary factors is involved in the operation. The only way that the balance of genes is involved is that an hereditary constitution is present which gives the various sex reactions in the various environments. The environments are the determiners of the specific results. The same principles and results are in evidence in the case of dioecious species, as proved by numerous past experiments on such plants.

Various oddities in sex mosaics sometimes appear. In one case, in addition to two normal carpellate spikelets at the base of the tassel, there was a spikelet with a typical, expanded carpellate base and typical, short, thickened glumes, but instead of a gynecium there

were eight normally developed stamens. In this case there was a rapid swing of the sex balance in the determinate spikelet axis from femaleness to maleness, just as when the stamen of a hemp flower suddenly reverses to femaleness and develops a typical stigma at its tip.

Occasionally an unbranched tassel mosaic of five reaction zones is expressed, with femaleness at the base, next a neuter zone, a male zone in the middle, followed by a second neuter zone, and femaleness at the tip again. Sometimes the neuter zones between the normally expressed sexual areas are very narrow or practically absent. If the neutrality of the intercalated neutral zones is extreme enough to inhibit stamen development but still permit development of glumes with partial, secondary male characters, then there is often a decided reaction toward internodal development. Thus with the proper balance, one or more prominent internodes may separate the compact, vestigial, neutral portion above and the compact, female portion below; or in case of a triple mosaic, with female expression below and above and male expression in the middle, the three sex zones may be separated by neutral zones with prominently developed internodes.

In our ordinary varieties of corn, the lowest side branches or suckers usually develop a functional gradient which causes sex reactions to appear in the same manner as in the main stem; but in general, the higher from the base of the plant the sucker originates the more decidedly female it becomes. A sucker developed several internodes up from the base usually produces an ear or a stamen-carpel mosaic at the tip, even in a long light period. With shortening of the light period even the lowest suckers develop ears at the tip, as would be expected.

NEUTRAL TASSELS

It must be remembered that all corn populations will give fluctuation series in respect to sexual expression. This is true whether the variety of corn used is an ordinary commercial variety or a highly inbred line. It will be exceedingly difficult, therefore, to produce an extreme percentage of neutral tassels in a given population, because physiological and morphological fluctuations are already present in the seed when planted and must be continued, and because it is im-

possible to give the growing individuals absolutely the same ecological environment. On the other hand, since the physiological state can be so manipulated that all of the individuals are passing their terminal systems into the female state before reproductive differentiation takes place, and since these physiological states can be continued in the same directions, it is easily possible to bring all of the tassels to a greater or less degree of carpellateness; and probably, with advanced knowledge in ecological control, even to the point of every individual having a respectable ear where the tassel should be. This should be done in the end as easily as keeping the population in an environment where all the tassels develop normally as staminate systems. With proper length and intensity of daily illumination, 40 per cent or more of the population can be induced to have completely neutral, vestigial tassels, with an additional number of nearly neutral tassels.

If one is depending on the changing daylight periods of the different seasons, the largest percentage of completely neutral tassels will be developed in plantings made between the first of August and the first of October. In the original experiments with sex reversal in corn, made several years ago, no separate records were kept of the neutral tassels developing, because at the time the chief interest was centered in the reversal from maleness to normal femaleness. It was therefore necessary to carry on an entirely new series of experiments to determine the rate of production of neuters.

The more recent studies with Narrow-grain Evergreen Sweet corn give the statistical results shown in table I. The depth of soil on the benches was mostly about 8 inches, and manure and abundant water were supplied. This table shows that all the neutral tassels were produced on a decreasing photoperiodic gradient between August 1 and November 1, with two exceptions, one on November 10 and one on January 1. In the first of the August 1 plantings recorded in the table as planted out of doors, although no completely sterile tassels developed, there were nevertheless three with decidedly sterile branches. The tassels which were entirely neutral except for one basal ovulary and silk were not counted, since these were included with those individuals which had undergone reversal from maleness to femaleness in part of the tassel.

The percentages of neuters of the different plantings show considerable fluctuation, as would be expected. This is probably due to the diversity of habitat conditions, since, as intimated before, these

TABLE I
PERCENTAGE OF NEUTRAL TASSELS PRODUCED AT DIFFERENT
PLANTING DATES FROM 1927 TO 1929; NARROW-GRAIN
EVERGREEN SWEET VARIETY

TIME OF PLANTING		NUMBER OF PLANTS	NUMBER OF COMPLETELY NEUTRAL TASSELS	PERCENTAGE OF NEUTRAL TASSELS
January	1.....	32	1	3
"	2.....	21	0	
"	20.....	15	0	
February	2.....	27	0	
"	15.....	38	0	
"	16.....	30	0	
March	1.....	27	0	
"	15.....	45	0	
"	21.....	34	0	
April	1.....	46	0	
"	1.....	49	0	
"	15.....	59	0	
"	16.....	20	0	
May	15*	228	0	
June	14.....	11	0	
July	1*	224	0	
"	2*	40	0	
"	2*	295	0	
"	15.....	32	0	
August	1*	60	0	
August	1.....	53	10	18
"	15.....	45	8	17
September	1.....	50	4	8
"	15.....	57	13	22
October	10†.....	22	9	41
"	10.....	29	4	13
"	15.....	20	1	5
"	17.....	26	1	4
"	29.....	31	1	3
November	1.....	20	0	
"	1.....	30	0	
"	10.....	31	1	3
"	15.....	25	0	
"	15.....	31	0	
December	1.....	28	0	
"	15.....	71	0	

* Planted in garden out of doors.

† Low light intensity.

could not be made very uniform. The comparative amount of cloudiness and sunshine may also have considerable effect. With a greater environmental uniformity for the various plots, as well as for the individuals of the plot, the percentages would no doubt show more regular conformity to the length-of-daylight factor, especially if large populations were employed.

A plot of forty-two plants of Early Connecticut 14-4, inbred for ten generations, planted November 1, 1928, showed two completely neutral tassels and forty with some reversal to femaleness varying from one silk and ovulary in the tassel up to forty-three.

FLUCTUATION SERIES IN REVERSAL TO FEMALENESS

Influence of the length of daily illumination, extending from about August 1 to about April 1, results in a remarkable fluctuation series in the reversal to female expression in the tassel, the reversal consistently increasing from a mere trace of femaleness in the terminal tassel at the beginning of this period to about 100 per cent by about November 1, followed by a gradual decrease to normal or nearly normal male expression by April 1. Not only is there fluctuation of the individuals of the given population, but the average number of ovaries and silks produced shows an increase from zero to about 35 for the shortest photoperiodicity. These results are summarized in table II.

INDIVIDUAL FLUCTUATION

Tables III-V show the remarkable difference in reaction of individual plants at three selected dates, August 15, October 29, and February 16. Study of a complete series of such tables enormously impresses one with the extreme differences of reaction under the various illumination periods, not only by the plots as a whole but by the individuals in the plots. As will be shown later, exactly similar results are produced in populations of highly inbred lines. The mechanism of sex determination and expression is thus plainly a matter of physiological conditions and functional gradient in the cells and tissues involved, and these physiological conditions are determined through the interaction of the hereditary constitution of the individual with the environment.

FLUCTUATION SERIES OF INDIVIDUALS SHOWING MAXIMUM
PRODUCTION OF SILKS AND OVULARIES, JULY TO APRIL

There is a general progression in the number of silks produced on the individual showing the greatest extreme of sex reversal to the

TABLE II

COMPLETE RECORD OF REVERSAL TO FEMALENESS IN TASSEL OF PLANTINGS FOR
1927-1929; NARROW-GRAIN EVERGREEN SWEET CORN (NEUTRAL
TASSELS COUNTED AS NOT REVERSED)

DATE OF PLANTING	TOTAL PLANTS	TASSEL NOT REVERSED	SEX REVERSAL IN TASSEL	PERCENT- AGE REVERSAL	TOTAL NO. OF SILKS IN TASSELS	AVERAGE NO. OF SILKS PER INDIVIDUAL OF POPULATION
May 15*	228	228	0			
June 14	11	11	0		
July 1*	224	224	0			
" 2*	40	40	0			
" 2*	295	294	1		1	
" 15	32	32	0			
August 1*	60	59	1	1 1/3	22	1/3
" 1	53	51	2	3 1/2	46	9/10
" 15	45	36	9	20	125	2 7/10
September 1	50	34	16	32	261	5 2/10
" 15	57	34	23	40	212	3 5/7
" 19	25	15	10	40	94	3 4/5
October 10†	22	9	13	59	174	8
" 10	29	4	25	86	418	14 2/5
" 15	20	1	19	95	581	29
" 17	26	4	22	84 1/2	467	18
" 29	31	2	29	93 1/2	685	22
November 1‡	20	0	20	100		
" 1	30	1	29	96 2/3	776	26
" 15	31	3	28	90 1/3	993	32
" 15	25	2	23	92	877	35
December 1	28	3	25	89 1/3	400	14
" 15	71	15	56	78	887	12 1/2
January 1	32	16	16	50	54	1 2/3
" 2	21	5	16	76	365	17
February 2	27	12	15	55 1/2	78	3
" 15	38	28	10	26 1/3	19	1/2
" 16	30	24	6	20	25	5/6
March 1	27	24	3	11	9	1/3
" 15	45	41	4	9	19	2/5
" 21	34	34	0			
April 1	46	44	2	4 1/2	13	1/3
" 1	49	48	1	2	1	1/50
" 15	59	59	0			
" 16	20	20	0			

* Planted in garden out of doors.

† In tank with 3 feet deep soil and poor light.

‡ Individual silks not counted, planting of 1926.

maximum date and then a decrease to the normal again. The number of silks in the tassel on extreme individuals was as follows: July 2, 0; July 2 (second plot), 1; August 1, 37; August 15, 50; September 1, 64; September 15, 106; October 10, 45; October 15, 154; October 17, 106; October 29, 105; November 1, 96; November 15, 146; November 15 (second plot), 142; December 1, 62; December 15, 128; January 1, 11; January 2, 92; February 2, 15; February 15, 4; February 16, 12; March 1, 5; March 15, 10; March 21, 0; April 1, 12; April 1 (second plot), 1; April 15, 0; April 16, 0.

TABLE III

NARROW-GRAIN EVERGREEN SWEET CORN
PLANTED AUGUST 15, 1928

NUMBER OF PLANTS	STAMINATE TASSELS	TASSELS WITH SEX REVERSAL	NUMBER OF SILKS PRODUCED
36	36
1	1	2
1	1	1
1	1	8
1	1	3
1	1	6
1	1	12
1	1	5
1	1	50
1	1	38
Total . . 45	36	9	125

Since the plots varied greatly in number of individuals and were comparatively small, no great regularity could be expected; but aside from this factor there are evidently numerous ecological and internal conditions which affect the reaction system. Nevertheless the reversal curve passes from zero to a maximum and then declines to zero again.

The common explanation advanced by those who still hold the assumption that a chromosome factor or set of factors determines sex, even though environment can change the sex ratio by changing the nutritive balance, is of no value because it merely adds another assumption to the primary one, that there were sex factors to determine the sexual states in the first place. So far as the writer can discover no one has ever offered any definite proof for the assump-

TABLE IV
NARROW-GRAIN EVERGREEN SWEET CORN
PLANTED OCTOBER 29, 1928

NUMBER OF PLANTS	STAMINATE TASSELS	TASSELS WITH SEX REVERSAL	NUMBER OF SILKS PRODUCED
2	2
I	I	1
I	I	4
I	I	27
I	I	25
I	I	73
I	I	5
I	I	15
I	I	10
I	I	22
I	I	5
I	I	10
I	I	13
I	I	20
I	I	105
I	I	3
I	I	2
I	I	2
I	I	22
I	I	2
I	I	5
I	I	4
I	I	62
I	I	52
I	I	17
I	I	4
I	I	8
I	I	76
I	I	40
I	I	42
Total .31	2	29	685

TABLE V
NARROW-GRAIN EVERGREEN SWEET CORN
PLANTED FEBRUARY 16, 1928

NUMBER OF PLANTS	STAMINATE TASSELS	TASSELS WITH SEX REVERSAL	NUMBER OF SILKS PRODUCED
24	24
I	I	1
I	I	1
I	I	3
I	I	1
I	I	7
I	I	12
Total .30	24	6	25

tion that the sex of an individual, a tissue, or a cell is determined by a sex gene or sex factor mechanism. Even in case differential allosomes are present in dioecious species, it is definitely known that, in case of reversal to the opposite sex no change in chromosome constitution takes place. If it were impossible to produce sex reversals and fluctuation sex series at will, and especially if no sex reversal could take place without a corresponding shift of allosomes, there might be some plausibility for a belief in the assumption; but since both monoecious and dioecious plants react regularly to produce fluctuation sex series and sex reversals, when placed in the proper environments, it seems impossible to reconcile the conflicting views on the subject.

REACTION OF PURE LINE VARIETIES TO PHOTOPERIODICITY

Plantings were made of two varieties of inbred Dent corn obtained from the Department of Farm Crops. They were Early Connecticut 14-4 and Late Connecticut 1-6. The Early Connecticut was inbred for ten generations and the Late Connecticut for thirteen generations. There was a marked difference in the vegetative growth of the two varieties. In general, when they were planted November 1, the Early Connecticut bloomed nearly a month earlier than the Late Connecticut. The long vegetative condition of the Late Connecticut during the short daylight period of winter also seemed to have a more decided influence in producing sex reversal, since when it came into full bloom by February 15 it showed 100 per cent with an average of $63 \frac{2}{5}$ silks per tassel (fig. 4), as compared with 95 per cent for the Early Connecticut with an average of only $8 \frac{2}{3}$ silks per tassel.

In tables VI and VII the striking differences in reaction of the two varieties are shown. The difference in sex reaction in different individuals is of the same nature as in the heterozygous commercial strain Narrow-grain Evergreen Sweet. The fluctuation series developed, therefore, is not at all due to a complexity of reacting sex genes, as some have imagined, but simply represents a functional reaction to diversity of environments, the change in functional state causing a greater or smaller tipping of the sex balance from maleness to femaleness in the tassel.

A plot of the inbred Late Connecticut 1-6 was planted February 1, 1929, in a sunny part of the greenhouse. It consisted of 19

individuals all normally developed, showing no reversal to femaleness in the tassel (fig. 4).

In 1927 Miss COLLET, a student in the department, carried on some experiments under the direction of Dr. A. E. WALLER on the influence of light on various phases of growth and maturity in corn.



FIG. 4.—Two plants of Connecticut Late 1-6, pure line: at left an individual planted November 1, showing complete female expression; at right an individual planted February 1, showing usual monoecious condition developed in long daylight period.

One of her experiments was also with Early Connecticut 14-4, which had been inbred nine generations. The planting of October 19 consisted of 36 plants in pots, all under extremely uniform conditions. All reversed their tassels to femaleness except one, which had a completely neutral tassel. The 35 plants with reversal to femaleness developed gynecia with silks in the tassel ranging from 6 to 143, which

was a much larger expression of femaleness than is shown by the individuals of table VI.

COMPLETE SUPPRESSION OF MALENESS

One of the remarkable results brought out by the experiments is the fact that a plot of corn can be developed with no male character

TABLE VI

EARLY CONNECTICUT 14-4 DENT CORN, INBRED TEN GENERATIONS, PLANTED
NOVEMBER 1, 1928, IN 8 IN. SOIL ON GREENHOUSE BENCH

NUMBER OF PLANTS	PLANTS PRODUCING SOME NORMAL STAMENS	NEUTER TASSELS	REVERSED TO CARPELLATE	NUMBER OF SILKS AND OVULARIES PER TASSEL*
1 ..	50 staminate spikelets	1	22
1. ..	1 staminate spikelet	1	1
2	No stamens	2
2	No stamens	2	1
3		3	2
6		6	3
4		4	4
5.	5	5
3.	3	6
2	2	7
2	2	8
2	2	9
1	1	10
1	1	11
1	1	13
1.	1	15
1.	1	16
1	1	17
1	1	19
1	1	22
1.	1	23
1	1	36
1	1	43
Total...44	2=5%	2=5%	40=95%	381

* Average silks per individual=8½.

expression whatever. Thus one plot planted October 10, 1928, with Narrow-grain Evergreen Sweet had 29 plants. Four of these had completely neutral tassels and the other 25 had tassels with varying degrees of neutral and female expression, the number of silks and ovularies produced per tassel varying from one to 45. Not a single

normal anther with pollen was produced. Maleness was completely repressed, the male structures all being vestigial, as is the case with female or carpellate plants of dioecious species generally, which either show complete suppression of male structures or only vestiges.

Another plot planted October 15, 1928, had 20 plants. One plant had a completely neutral tassel while the remaining 19 plants all

TABLE VII

LATE CONNECTICUT 1-6 DENT CORN, INBRED THIRTEEN GENERATIONS, PLANTED
NOVEMBER 1, 1928, IN 8 IN. SOIL ON GREENHOUSE BENCH

NUMBER OF PLANTS	PLANTS PRODUCING SOME NORMAL STAMENS	NEUTER TASSELS	REVERSED TO CARPELLATENESS	NUMBER OF SILKS AND OVULARIES PER TASSEL*
I	I		I	7
I.....	I		I	15
I.....	I		I	19
I.....	I		I	20
I.....	I		I	20
I.....	I		I	26
I.....	I		I	30
I.....	I		I	39
I.....	I		I	42
I.	O		I	50
I.....	O		I	54
I.....	I		I	65
I.....	I		I	71
I.....	O		I	74
I.....	O		I	78
I.....	O		I	79
I.....	O		I	80
I.....	I		I	91
I.....	O		I	94
I.....	O		I	95
I.....	O		I	99
I.	O		I	102
I.....	O		I	105
I.....	O		I	110
I.....	O		I	111
Total.. 25	12=48%		25=100%	1585

* Average silks per individual=63½.

showed reversal to femaleness, ranging in expression from one silk and ovulary per tassel to 154. Of these 19 plants only two had pollen-bearing stamens and two had several small stamens each but without pollen. Thus in this plot only two individuals out of the 20 developed normal male states and expressions. All plants of the two plots developed normal carpellate inflorescences or ears at the side.

It is thus evident that not only is there a fluctuation series of reversal to femaleness in the tassel, but there is also a fluctuation series in the degree of expression of maleness. Apparently the optimum light period for producing complete suppression of maleness is attained with plantings between October 5 and 15. On either side of this period the fluctuation progresses to normal male expression in July on the one hand, and normal male expression in April on the other.

It is evident that little value can be attached to the deductions from genetic studies carried on to discover hereditary potentialities unless proper account has been taken of the ecological-physiological control of hereditary expression. The expression of femaleness with the production of an ear at the tip of a stalk is just as much an expression of its fundamental hereditary potentiality as is the expression of maleness with the production of a tassel. So if one plants a grain of corn in a short light season and gets a stalk with an ear developed at the tip, as well as one at the side, then, using the current genetic terminology, it would be proper to insist that the hereditary nature of corn is such that "naturally" an ear is developed at the tip, but the "abnormal" light environment of summer changes the activity of the genes by disturbing the nutritive balance, and thus a tassel is produced abnormally where one was expecting an ear. Instead of speaking of "normal" hereditary expression it is better to think of "usual" expression under the usual environment.

Summary

1. The experiments here reported establish the fact that completely neutral, vestigial tassels can be developed at will in *Zea mays* by the simple control of environmental conditions, and that the natural photoperiodic gradient extending from August to November 1 will sometimes produce 40 per cent or more of neutral tassels in a population. These results suggest that the problem of sterility in plants, animals, and man is amenable to experimental analysis and remedy.

2. With proper photoperiodicity, seven general types of tassels can be developed as follows: three of pure expression—staminate (male), carpellate (female), and vestigial (neuter); and four sex mosaics—staminate-neuter, carpellate-neuter, staminate-carpellate, and staminate-carpellate-neuter.

3. With the decreasing photoperiodicity of autumn, femaleness is only expressed at the base of the tassel and the tip is always neuter, the staminate expression occurring in the middle region. With the increasing photoperiodicity of winter and early spring, femaleness may also be expressed at the tip of the tassel and its branches, or sometimes in such a way as to form a complex mosaic with carpellate spikelets scattered among the staminate ones.

4. Experiments with highly inbred varieties show that pure lines react to changed photoperiodicity in the same way as commercial heterozygous varieties, giving rise to complete sex reversals, neutral vestigial tassels, and all the possible types of sex mosaics, as also extreme fluctuation series between members of a plot and fluctuation between successive plots developed in light periods of different lengths.

5. Male expression can be suppressed completely in the monoecious *Zea mays* when it is grown in a decreasing photoperiodic environment of suitable length. Entire plots of individuals can be developed without the appearance of a single stamen.

6. The diversity of sexual expression obtained is absolutely due to the diversity of physiological conditions produced through the diversity of environments, and has nothing to do in general with any diversity of hereditary factors which might be present in the different individuals of a given plot, since pure lines show just as extreme fluctuation in sex reaction as heterozygous lines. The specific sex condition developed is not dependent on any balance of sex-determining or sex-producing genes.

7. It is evident that genetic experiments involving sex conditions are of no value unless the reactions obtained are interpreted in the light of ecological conditions present.

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IMPERFECT SEXUAL REACTIONS IN HOMOTHALLIC AND HETEROTHALLIC MUCORS¹

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(WITH FIFTEEN FIGURES)

In a recent paper (8) various criteria of male and female in *Mucor* were discussed. It is the purpose of the present paper to give the detailed data upon which the earlier publication was based.

The imperfect sexual reaction which may take place between the (+) and (−) sexual races of different heterothallic species of *Mucors* has already been extensively studied (1, 3, 5). Such an interaction between sexes, which is illustrated in fig. 1, enables one to determine the sex of a given race which could not otherwise be so classified. The imperfect sexual reaction indicates the presence of some common factor which elicits the sexual activity between species which may not be closely related taxonomically, and which may even belong to different genera and families. This factor would seem to be qualitatively different from the one immediately responsible for the fusion in the perfect sexual reaction.

Earlier studies showed that contrasts between homothallic and heterothallic species give also imperfect sexual reactions (2). Different homothallic species, however, were found not to behave in the same way when contrasted with heterothallic races. Some of them reacted with both (+) and (−) races (*Mucor genevensis*), some (*Sporodinia grandis*) did not show any reaction, while other species showed either a (−) tendency (*Absidia spinosa*, *Zygorhynchus moelleri*, *Z. vuillemini*) and reacted predominantly with the (+) races or had a (+) tendency (*Z. heterogamus*) and showed reactions only with the (−) races.

In the homothallic species with the (−) tendency it was the small

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progametangia which reacted with the (+) races. In the homothallic species with the (+) tendency (*Z. heterogamus*), however, the small progametangia appeared to react with the (−) races. The different behavior of *Z. heterogamus* from that of the other heterogamic hermaphrodites seemed to indicate that the smaller gametangium of this species is (+) whereas the smaller gametangium of the other heterogamic hermaphrodites studied is (−). That the smaller gametangium in one species was (−) and in another species was (+) would show that the generally accepted belief in the relative size of uniting sex cells as a criterion of male and female cannot be of universal application.

Other phenomena in *Mucors* have shown that HARTMANN's (6) theory of relative sexuality cannot hold for this group. The matter seemed to be of sufficient importance, not only to an understanding of sexuality in *Mucors*, but also for the theories of sex in general, to warrant a more intensive investigation. For these reasons the studies reported in the present paper were undertaken. Specifically answers were sought to the following questions: (1) Is the (+) and (−) tendency in the hermaphrodites a specific or a racial property? (2) Are the terminal and lateral hyphae in species of *Zygorhynchus* bisexual in nature, and does the sexual plasm become separated in the gametangia and suspensors of this genus? (3) Which of the progametangium, small or large, in *Z. heterogamus* reacts with the (−) races and which with the (+) races?

The technique employed is the same as that given in a previous paper (4). It was absolutely necessary to make the observations in dishes in which channels were cut in the nutrient agar between the contrasted races.

Twenty homothallic races, included in eight species and five genera, were contrasted with seven (+) and six (−) sexually strong heterothallic races. Table I shows the results obtained from these contrasts. Both races of *Mucor genevensis* gave a strong reaction with all (+) and (−) races tested with one exception (*Absidia caerulea* (−)). *Sporodinia* did not react with any tester. A number of other heterothallic and homothallic species were contrasted with *Sporodinia* under various external conditions, but always failed to show any imperfect reaction. BLAKESLEE (1) and NIELSEN (7)

have obtained the same negative results. One race of *Dicranophora* sp., one race of *Absidia spinosa*,² and eleven races of *Zygorhynchus* included in three species (five races of *Z. moelleri* (971, 78, 932, 1007, 1004), two races of *Z. vuillemini* (one of them agamic), four races of *Zygorhynchus* sp. (1008, 1009, 931, 1010), an American species closely related to *Z. moelleri*), all showed a marked (—) tendency and reacted with the (+) races only, except for three cases in which few

TABLE I
REACTIONS BETWEEN HOMOTHALLIC AND HETEROTHALLIC SPECIES

HETEROTHALLIC RACES	HOMOTHALLIC RACES																			
	M. geneven. I	M. geneven. II	<i>Absidia spinosa</i>	<i>Dicranophora</i> sp.	<i>Zyg. moelleri</i> 971	<i>Zyg. moelleri</i> 78	<i>Zyg. moelleri</i> 932	<i>Zyg. moelleri</i> 1007	<i>Zyg. moelleri</i> 1004	<i>Zygor. sp.</i> 1008	<i>Zygor. sp.</i> 1009	<i>Zygor. sp.</i> 931	<i>Zygor. sp.</i> 1010	<i>Zyg. vuillemini</i>	<i>Z. vuil. var. agamus</i>	<i>Zyg. heterogam.</i> 1011	<i>Zyg. heterogam.</i> 934	<i>Zyg. heterogam.</i> 1012	<i>Zyg. heterogam.</i> 935	<i>Sporodinia</i>
(+) TESTERS																				
<i>Mucor</i> H.	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	O	O	O	O	O
<i>M. dispersus</i>	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	O	O	O	O	O
<i>Mucor</i> V.	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	O	O	O	O	O
<i>Rhiz. nigricans</i>	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	O	O	O	O	O
<i>Abs. caerulea</i> ..	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	O	O	O	O	O
<i>Abs. glauca</i>	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	O	O	O	O	O
<i>Abs. repens</i> ..	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	O	O	O	O	O
(-) TESTERS																				
<i>M. dispersus</i>	X	X	O	O	O	O	O	O	O	O	O	O	O	O	O	X	X	X	X	O
<i>Mucor</i> V.	X	X	X	O	O	O	O	O	O	O	O	O	O	O	O	X	X	X	X	O
<i>Rhiz. nigricans</i>	X	X	O	O	O	O	O	O	O	O	O	O	O	O	O	X	X	X	X	O
<i>Abs. caerulea</i> ..	X	X	O	O	O	O	O	O	O	O	O	O	O	O	O	X	X	X	X	O
<i>Abs. glauca</i>	X	X	O	O	O	O	O	O	O	X	O	X	O	O	O	X	X	X	X	O
<i>Abs. repens</i>	X	X	O	O	O	O	O	O	O	O	O	O	O	O	O	X	X	X	X	O

* X indicates imperfect sexual reaction; O indicates no reaction.

reacting progametangia were found in contrasts with (—) races (*Zygorhynchus* 1008 × *Absidia glauca* (—), *Zygorhynchus* 931 × *A. glauca* (—), and *A. spinosa* × *Mucor* V (—)). Imperfect reactions between *A. spinosa* and both (+) and (—) races of *Mucor* V were

² Certain earlier tests and statements regarding species with (—) tendency (*Z. moelleri*, *Z. vuillemini*, and *A. spinosa*) made by BLAKESLEE (2) apparently have been misinterpreted by BURGEFF (5). No reactions between these hermaphrodites and (—) races have earlier been reported by one of us, as BURGEFF (5) writes, except for one case (*A. spinosa* × *Mucor* V (—)). We considered them to be predominantly (—) or to have a (—) tendency because their small gametangia gave a strong sexual reaction with (+) testers, but no reaction or at best only a weak one with (—) testers.

observed in 1912 (2). These three races have been kept in cultivation since that time and now give the same reactions as before. This constancy of sexual character is in accordance with all our experience with *Mucors*.

The four races of *Z. heterogamus* showed a (+) tendency, since they reacted with only the (−) races in all cases but one (*Zygorhynchus* 1011 × *A. repens* (+)).

Besides the contrasts shown in table I, many others were made. Two races selected from the group with the (−) tendency (*Dicranophora* and *Zygorhynchus* sp. 1008) and two races from the group which showed the (+) tendency (*Z. heterogamus* 1011 and 934) were contrasted with both (+) and (−) races of a number of other heterothallic species (*Mucor mucedo*, *M. hiemalis*, *M. griseocyanus*, *M. III*, *M. IV*, *M. VII*, *Absidia bulleri*, *A. glauca* (3 pairs), *Rhizopus nigricans* (5 pairs), *Cunninghamella bertholletiae*, *C. elegans*, *C. echinulata*, *C. blakesleeana*, *Blakesleea trispora*, *Choanephora cucurbitarum*, *Phycomyces blakesleeanus*, *Circinella umbellata*, *C. spinosa*, *Helicostylum piriforme*, *Syncephalastrum racemosum*). There were some species both sexes of which failed to react with the selected races of the hermaphrodites. When any reaction was observed it was in accord with the sexual tendency of the hermaphrodites shown in table I. Without going into details, a brief summary of the tests made with *Dicranophora* may be given here.

Fifty-two different races representing 20 different species were used in this series. Of the 26 (+) races, 15 gave a distinct imperfect reaction with *Dicranophora* with the production of progametangia; five induced the formation of a distinct yellowish line with the hyphae surrounded by twisted filaments of *Dicranophora* but no progametangia were evident; six races showed no sexual reaction with the hermaphrodite. Of the 26 (−) races, 23 failed to show any sexual reaction with *Dicranophora* and three induced the formation of a narrow yellowish line but without other suggestion of a sexual reaction.

Table II shows the results obtained from contrasts between hermaphrodites. Zygosporos were obtained when the contrasted races belonged to the same species. Distinct imperfect sexual reactions were obtained between races of different species which showed op-

posite sexual tendencies. Thus *Absidia spinosa* which has a (−) tendency gives an imperfect reaction with the races of *Zygorhynchus heterogamus* which have a (+) tendency. On the contrary no imperfect reactions were observed between races of species which have the same sexual tendency. *A. spinosa*, for example, which has a (−) tendency, has shown no imperfect reaction with the races of *Z. moelleri* or *Z. vuillemini* and *Dicranophora* which have also a (−) tendency. So far as our experience has gone, the individual races of a

TABLE II
REACTIONS BETWEEN HOMOTHALLIC SPECIES

	SPECIES STRONGLY (+) & (−)	SPECIES PREDOMINANTLY (−)				SPECIES PREDOMINANTLY (+)	
		Mucor genev.	Dicranophora	Absidia spinosa	Z. species 1008	Z. heterogamus 934	Z. heterogamus 1011
SPECIES PREDOMINANTLY (−)	Dicranophora	X*	Z	O	O	X	X
	Abs. spinosa	X	O	Z	O	X	X
	Zygorhynchus sp 1008	X	O	O	Z	X	X
	Zygorhynchus sp. 931.	X	O	O	Z	X	X
	Zygorhynchus moel. 78	X	O	O	O	O	O
	Zygorhynchus moel. 932	X	O	O	O	O	O
	Z. vuillemini	X	O	O	O	O	O
SPECIES PRE- DOMINANTLY (+)	Z. heterogamus 1011	X	X	X	X	Z	Z
	Z. heterogamus 934	X	X	X	X	Z	Z
	Z. heterogamus 1012	X	X	X	O	Z	Z
	Z. heterogamus 935 ..	X	X	X	X	Z	Z

* Z indicates the production of zygospores; X imperfect sexual reaction; O no reaction.

given heterogamic species have had the same sexual tendency. Hermaphroditic species with a (−) tendency are more frequent than those with a (+) tendency. In contrast to the forms discussed, *Mucor genevensis* has both sexual tendencies sufficiently developed to give imperfect reactions with both the predominantly (+) and the predominantly (−) groups.

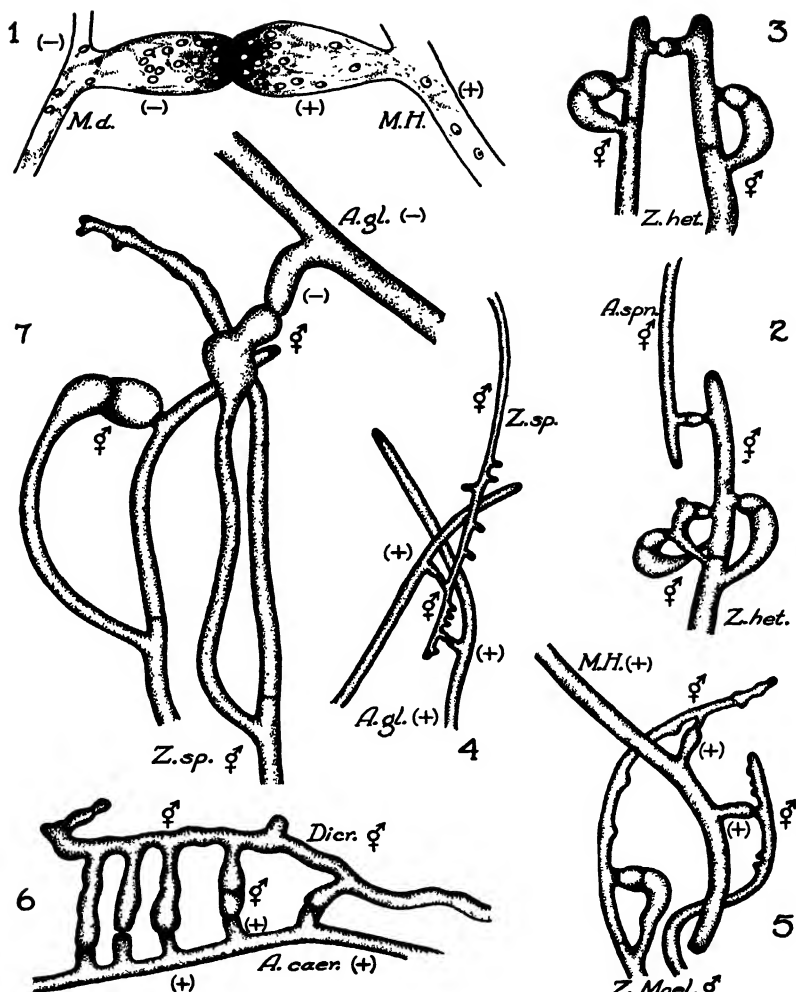
In the perfect sexual reaction which brings about the formation of zygospores there is a morphological differentiation of the zygothoracic hyphae. A slender terminal hypha usually forms the smaller game-

tangium, while the larger gametangium is usually developed from a swollen lateral branch. A number of lines of evidence show that despite the morphological differentiation of the two progametangia, neither of them is strictly of a single sex. For example, a large progametangium or suspensor may develop a small gametangium (fig. 2) in which three perfect reactions may be observed, the large progametangia of which have arisen close together from an erect hypha. In one of these three, however, the small gametangium may be seen perched on the back of what is now a large suspensor. In fig. 3 are shown again three perfect reactions, two of them of the usual type but the third forming a zygospor between the two filaments, with the large progametangium therefore produced by a terminal hypha. Both of these figures are from *Zygorhynchus heterogamus*.

It has frequently been found that terminal zygophoric branches may differentiate lateral branches and that lateral ones may give rise to terminal branches. Tests have further shown that both lateral and terminal zygophoric hyphae can develop sporangia with bisexual spores. This observation confirms similar results obtained in *Z. exponens* by BURGEFF (5). In some cases the differentiation of lateral branches may fail altogether and perfect reactions occur between the terminations of elongated hyphae. All these cases lead to the conclusion that both zygophoric branches in young or old stages are bisexual. NIELSON (7), working with *Absidia spinosa*, has reached the same conclusion.

Further experimental studies on *Dicranophora* (regeneration of isolated progametangia), which may be published later, strengthen the belief that the plasm in progametangia of hermaphrodites is undifferentiated sexually; but there is no experimental evidence available regarding the sexual condition in the gametangia themselves.

The lack of sharp localization of sex in the zygophoric hyphae of these hermaphrodites may account for the abundant production of what appear to be small progametangia along the whole length of hyphae when the hermaphrodite is grown in contrast with a race with which it shows an imperfect reaction. Thus in figs. 4, 5, and 11, in which imperfect reactions may be observed, the hyphae of the



FIGS. 1-7.*—Fig. 1, imperfect reaction between *Mucor* H (+) and *M. dispersus* (-) (note dense plasm and crowded nuclei at line of contact between both progametangia); cell wall between the two sex cells remains intact. Fig. 2, below: three perfect sexual reactions in *Zygorhynchus heterogamus* 934; small gametangium developed on suspensor of large gametangium and taking part in perfect reaction; above: imperfect reaction between small progametangium of *Zygorhynchus* and small gametangium of *Absidia spinosa*. Fig. 3, three perfect sexual reactions in *Z. heterogamus* 1011; in one of them both large and small gametangia developed from terminal hyphae. Fig. 4, two imperfect reactions between small progametangia of *Zygorhynchus* sp. 1008 and *A. glauca* (+) (note abundant wartlike projections along length of hyphae of *Zygorhynchus*). Fig. 5, two imperfect reactions between small progametangia of *Z. moelleri* 1004 and *Mucor* H (+); abundant wartlike projections on hyphae of hermaphrodite. Fig. 6, five imperfect reactions between small progametangia of *Dicranophora* sp. and *A. caerulea* (+). Fig. 7, imperfect reaction between large progametangium of *Zygorhynchus* sp. 1008 and *A. glauca* (-); large progametangium developed from lateral hypha; compare with perfect reaction at left of figure.

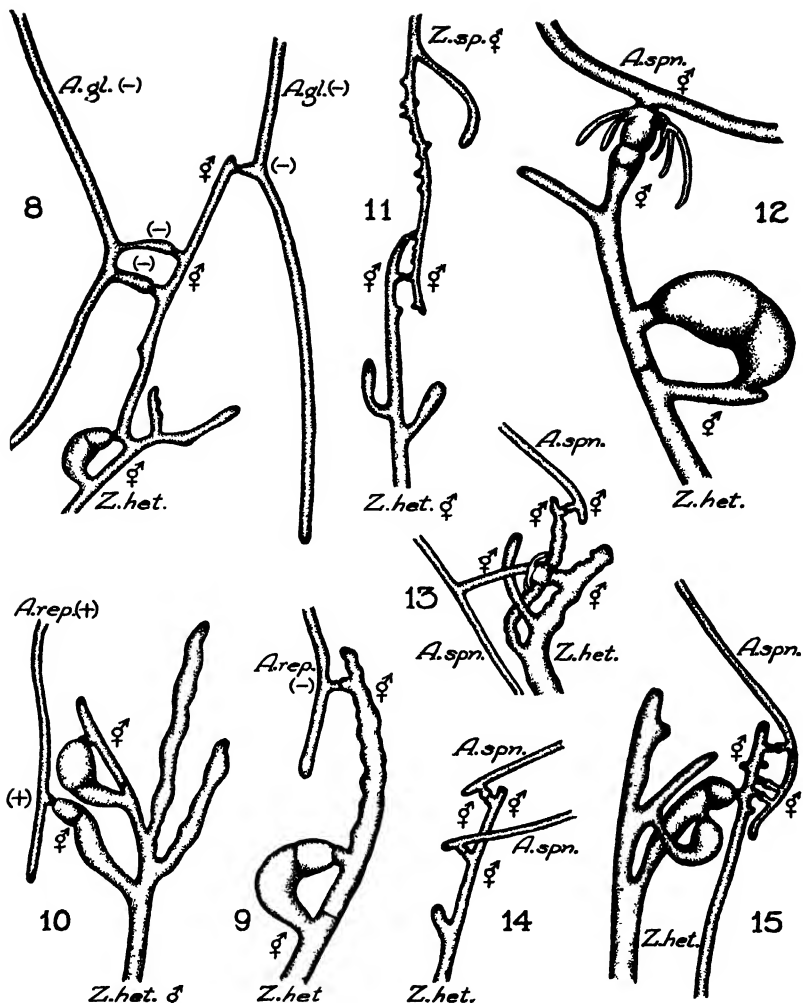
* All figures drawn with aid of Abbe camera lucida: fig. 1 from fixed and mounted material under Leitz 2 mm. apochromatic objective combined with compens. ocular 15X; figs. 2-6, 8-11, 13-15 from living material under Zeiss objective 8 and compens. ocular 15X; figs. 7 and 12 from living material under Zeiss objective 20 and compens. ocular 15X.

hermaphrodites are covered with small warts resembling small progametangia but unconnected with filaments of the other species responsible for the imperfect reactions. Such wartlike projections in most species are rare when the hermaphrodites are grown in pure cultures. On stout lateral branches they do not appear.

Figs. 4-6 illustrate imperfect reactions between the species with a (-) tendency (*Zygorhynchus* sp., *Z. moelleri*, and *Dicranophora*) on the one hand and (+) races of heterothallic species (*Absidia glauca*, *Mucor* H, and *A. caerulea*) on the other hand. In all cases it is apparently the small progametangium of the hermaphrodite which reacts with a progametangium of the (+) tester. The condition is strikingly different in those rare cases shown in fig. 7, in which a reaction takes place between one of these hermaphrodites and a (-) race of a heterothallic species. In figs. 4 and 7 the same race of the hermaphrodite (*Zygorhynchus* 1008) is represented but the opposite sexes of the heterothallic species ((+) and (-) *A. glauca*). It is evident from these figures and from other observations on this group of heterogamic species of *Mucors* that the (+) testers react with the small progametangium while the (-) testers react with the large progametangium.

Special attention was given to a study of the character of the reacting cells in *Z. heterogamus* which belongs to a group with a different sexual tendency. This hermaphrodite behaves in a manner the exact opposite of that shown by the other hermaphrodites just discussed. It shows strong reactions with (-) instead of with (+) testers and weak or no reactions with (+) instead of with (-) testers. Moreover, a (-) tester stimulates a reaction in its small rather than in its large progametangium (figs. 8, 9). In figs. 9 and 10 the same race of the hermaphrodite (*Z. heterogamus* 934) is represented in reaction with the (-) and (+) races respectively of *Absidia repens*. It is evident that the large progametangium reacts with the (+) and the small progametangium with the (-) race of the heterothallic *Absidia*.

The reaction shown in fig. 10 is the only conclusive one observed out of numerous contrasts with a considerable number of (+) races from many different species. A few doubtful cases have been recorded but lack of any sexual reaction is characteristic of the contrast between *Z. heterogamus* and (+) races.



FIGS. 8-15.—Fig. 8, three imperfect reactions between small progametangia of *Zygoryhynchus heterogamus* 1011 and *Absidia glauca* (—); compare size and shape of these small progametangia with small sexual cell in perfect reaction in lower part of figure. Fig. 9, imperfect reaction between small gametangium of *Z. heterogamus* 934 and *A. repens* (—); perfect reaction is shown in lower part of figure. Fig. 10, imperfect reaction between large progametangium of *Z. heterogamus* 934 and *A. repens* (+); perfect reaction shown above. Fig. 11, two imperfect reactions between small progametangia of *Z. heterogamus* 1011 and small progametangia of *Zygoryhynchus* sp. 1008; hyphae of latter species covered with wartlike projections. Fig. 12, imperfect reaction between large gametangium of *Z. heterogamus* 1011 and large gametangium of *A. spinosa* (note circinate outgrowths from suspensor of *A. spinosa*); perfect reaction of *Zygoryhynchus* shown in lower part of figure. Fig. 13, two imperfect reactions between *Z. heterogamus* 1011 and *A. spinosa*; in middle of figure the reaction is between two large gametangia, that of *Absidia* being subtended by circinate outgrowths; in upper part of figure the reaction is between two small progametangia. Fig. 14, two imperfect reactions between small progametangia of *Z. heterogamus* 934 and small progametangia of *A. spinosa*. Fig. 15, three imperfect reactions between small progametangia of *Z. heterogamus* 934 and small progametangia of *A. spinosa*; two perfect reactions shown in *Zygoryhynchus* in one of which the small gametangium developed from a large suspensor.

In view of the almost complete failure to obtain sexual reactions with the large progametangium of *Z. heterogamus*, studies were made of the reactions between *Z. heterogamus* and other hermaphrodites (*Absidia spinosa*, *Zygorhynchus* sp.). From the reactions shown with (+) and (-) races of heterothallic species, we may consider in *Z. heterogamus* the small gametangium as (+) and the large one as

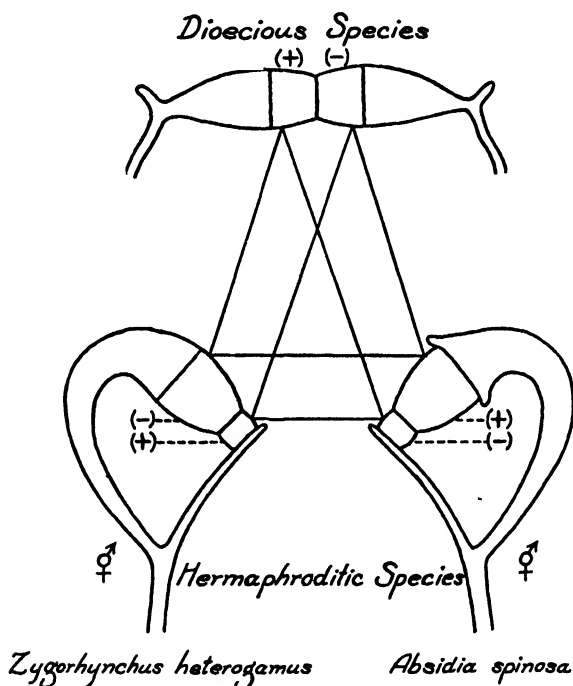


Diagram showing by solid lines the imperfect reactions obtained between a dioecious species and two types of heterogamic hermaphrodites; in one hermaphrodite the larger sex cell is (+) while in the other it is (-).

(-). In *A. spinosa* and the other heterogamic hermaphrodites, however, the small gametangium is (-) and the large gametangium (+).

If the interpretation just made concerning the sexual nature of the large and small gametangia in these forms is correct, contrasts between *Z. heterogamus* and the other hermaphrodites (*Z. moelleri* type, *A. spinosa*, *Dicranophora*) should bring about a reaction between a small (+) gametangium of *Z. heterogamus* and a small (-)

gametangium of the other hermaphrodites. There would also be expected a reaction between a large (−) gametangium of *Z. heterogamus* and a large (+) gametangium of the other hermaphrodites mentioned. This expectation was fulfilled, as shown in the adjacent diagram. Fig. 11 illustrates a reaction between small progametangia of *Z. heterogamus* and small progametangia of *Zygorhynchus* sp. 1008. Figs. 12–15 show the expected reactions between *Z. heterogamus* and *A. spinosa*. *A. spinosa* was especially adapted to such contrasts because of the presence of circinate outgrowths from suspensors on the side of the large (+) gametangium and absence of such outgrowths below the small (−) gametangium. Successive observations made during several hours on the same reacting progametangia permit definite conclusions concerning the size of the sex cells in *Absidia* and in *Zygorhynchus* with which it reacts. Fig. 12 shows a reaction between a large gametangium of *Z. heterogamus* which had developed on a lateral hypha, and a large gametangium of *A. spinosa*. A similar reaction can be seen in fig. 13, in which the large (+) gametangium of *A. spinosa* reacts with a large (−) progametangium of *Z. heterogamus*. The progametangium of the latter species arose on a thick hypha and is less well developed than the one shown in fig. 12. Fig. 13 shows also a reaction between the small progametangia of the two contrasted hermaphrodites. In this figure a comparison can readily be made between the sizes of the reacting sex cells. In both figs. 12 and 13 the large gametangia of *A. spinosa* may be seen to be subtended by the circinate outgrowths characteristic of this species. Figs. 14 and 15 show reactions between small (+) progametangia of *Z. heterogamus* and the small (−) progametangia of *A. spinosa*. There were seven small outgrowths that had developed from a hypha of *Z. heterogamus* (fig. 15). Three of them reacted with *A. spinosa*, three are free, and one is involved in a perfect reaction with a large gametangium from a neighboring hypha. The suspensor of this latter gametangium has given rise to a small gametangium also involved in a perfect reaction (cf. also fig. 2).

In an earlier paper (8) we discussed the various criteria of male and female in Mucors and the relations which the findings reported in more detail in the present paper may have with other sexual phenomena. It will not be necessary, therefore, to enter into a further

discussion of these matters here. It will suffice if we have presented adequate data in support of our belief that in the heterogamic hermaphrodite *Zygorhynchus heterogamus* the large and small gametangia give an opposite sexual reaction from that given by the large and small gametangia of the other heterogamic hermaphrodites.

Summary

Imperfect sexual reactions were tested between homothallic species (including both homogamic and heterogamic forms) and (+) and (−) races of heterothallic species. Among the heterogamic hermaphrodites there are two sexual groups: (1) those with a (−) sexual tendency (*A. spinosa*, *Dicranophora*, *Z. moelleri*, *Z. vuillemini*, *Zygorhynchus* sp.); these show strong reactions with (+) races and weak or no reactions with (−) races; and (2) those with a (+) tendency (*Z. heterogamus*). These show strong reactions with (−) races and weak or no reactions with (+) races. So far as investigated, all the races of a given species have the same sexual tendency.

The terminal and lateral zygophoric hyphae of the hermaphrodites investigated are bisexual. The terminal hyphae take part more actively in imperfect sexual reactions than do the lateral branches.

In the group of hermaphrodites with a (−) tendency, the large gametangium is shown to be (+) from its reactions with (−) races and the small gametangium is shown to be (−) from the reaction with (+) races. In the group with a (+) tendency, represented by *Z. heterogamus*, however, the large gametangium is shown to be (−) in reaction and the small gametangium to be (+). Representative hermaphrodites of these groups when grown together have given imperfect reactions. A small gametangium of the one group reacts with a small gametangium from the representative of the other group, or less frequently two large gametangia, one from each group, show a mutual reaction.

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A PARTIAL REVISION OF FOSSIL FORMS OF ARTOCARPUS

OSCAR M. BALL

(WITH SEVENTEEN FIGURES)

Introduction

Fossil remains assigned by investigators to *Artocarpus* first appear in the Cretaceous of Greenland. They were described by NATHORST (12) and consisted both of leaves and of parts of the fruit. About twenty-five years earlier, however, LESQUEREUX had described from the Laramie beds of Boulder County, Colorado, certain huge leaves as *Myrica* (?) *lessigniana* (9) and *Myrica* (?) *lessigii* (10). The latter specific name is apparently an error of LESQUEREUX'S. NATHORST pointed out that these leaves were in reality those of *Artocarpus*. KNOWLTON (5) transferred both of LESQUEREUX'S forms to *Artocarpus lessigniana* (Lesquereux) Knowlton.

LESQUEREUX (11) recorded from the Denver formation at Golden, Colorado, other leaves as *Aralia pungens*. Leaves similar to these were discovered by HOLLICK (4) in beds of the lower Eocene in Louisiana. Like NATHORST, he recognized their true affinity and recorded them as *Artocarpus pungens* (Lesquereux) Hollick. In addition, HOLLICK records a new species, *Artocarpus dubia*, from the same beds, a red sandstone, one-fourth of a mile above Couchatta. In his notes he states, "This may be merely a young or small leaf of *Artocarpus lessigniana* (Lesquereux) Knowlton." This surmise is correct. HOLLICK records *Artocarpus lessigniana* from beds near Shreveport in the same state. BERRY (2) redescribed these three "species" from various localities in the Embayment province, and made the same notation regarding *A. dubia* as that of HOLLICK already quoted.

KNOWLTON (6) has a species, *Artocarpus similis*, which he states is, "in a way, intermediate between *A. lessigniana* and *A. pungens*. . . . The differences separating the three are not very marked, and it is quite possible that with a fair series of specimens, they might

be found to be indistinguishable." This series has, I think, been found in the beds of the Indio of Texas. The leaf figured by KNOWLTON in pl. 78, fig. 1 has a base with deeply decurrent margins. This feature is a characteristic commonly found in specimens from the Bastrop County beds.

From the Raton field in southern Colorado KNOWLTON (7) described a fragment of a leaf from the Vermejo formation (Cretaceous) as *Artocarpus dissecta*. He notes its close resemblance to *A. lessigniana* and suggests that a series of leaves would show their identity. This fragment may be compared with one obtained from Barton's ranch (fig. 2) which is clearly *A. lessigniana*. It might be urged that *A. dissecta* Knowlton should be considered a true species because it appears in Cretaceous beds, and therefore is vastly older than the Eocene forms. In this connection it must be observed that the type locality of *A. lessigniana* is in the Laramie, at Coal Creek, which formation is usually placed in the Upper Cretaceous.

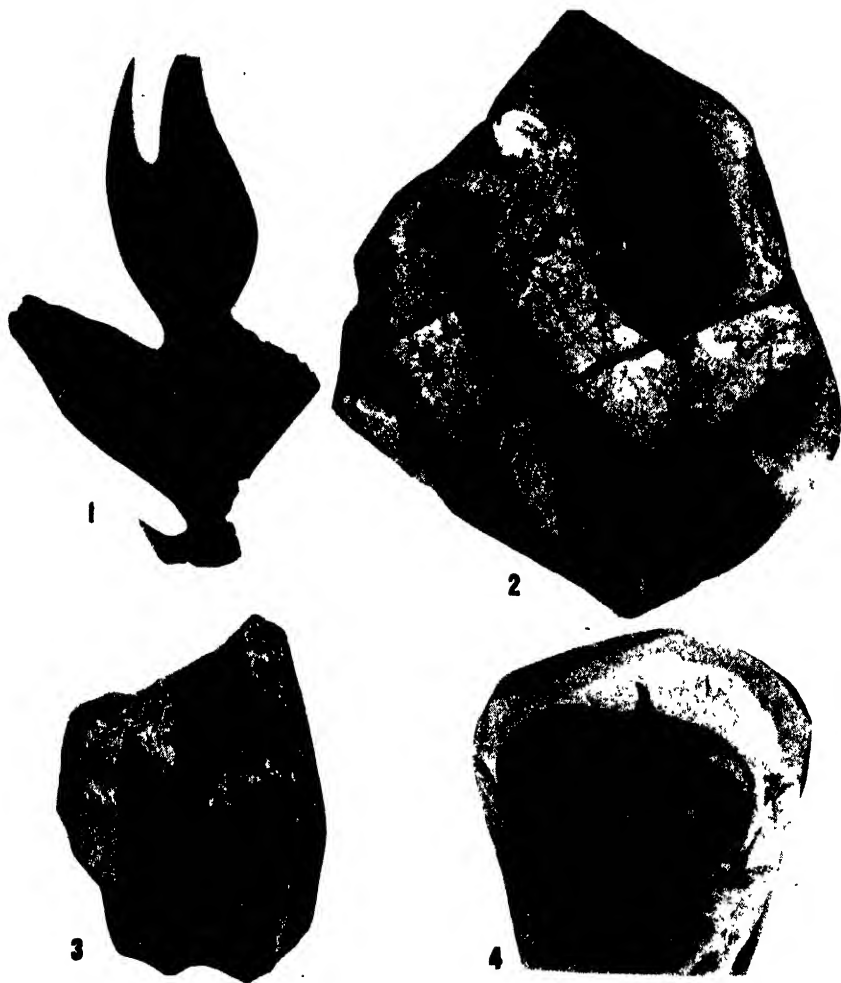
KNOWLTON (8) at one time combined *A. pungens* with *A. lessigniana*, and BERRY (2) mentions this fact in his redescription of *A. pungens*. KNOWLTON separated them again in his Raton flora, however, and BERRY (3) also separated them, but has since reached the conclusion that the two are identical and has indicated this view to me in correspondence.

Investigation

The writer has made extensive collections of fossil plants from beds of the Indio formation (Eocene) from two localities in Bastrop County, Texas. The first of these stations is on the ranch of Mr. John Barton, and is an exposure in the bank of a small tributary to Wilbarger Creek, about one-half mile north of bench mark 391, Bastrop quadrangle. Fifty-five species have been described (1) from these beds, which contained great numbers of individual specimens, among them a few fragments of *Artocarpus lessigniana*.

Exposures of the same formation appear in a bluff on Little Sandy Creek, about 4 miles due east of the Barton ranch station and 2 miles north of Sayers. At the time of this writing, 42 species have been recovered from these beds. They carry enormous numbers of the leaves of a fan-palm and an abundance of those of *Artocarpus*, of a great variety of form. One striking form of the latter has as yet

appeared nowhere else (figs. 6, 15). After a prolonged and exhaustive study of this new form it was referred to Professor BERRY, who



FIGS. 1-4.*—Fig. 1, *Aralia pungens* Lesquereux = *Artocarpus lessigniana* (Lesquereux) Knowlton; Golden, Colorado; original in Princeton University; fig. 2, *A. lessigniana* (Lesquereux) Knowlton; fig. 3, *A. lessigniana* (Lesquereux) Knowlton; *dubia* type; fig. 4, *A. lessigniana* (Lesquereux) Knowlton; aberrant form.

* All leaves except that of fig. 1 are from beds of the Indio formation in Bastrop County, Texas.



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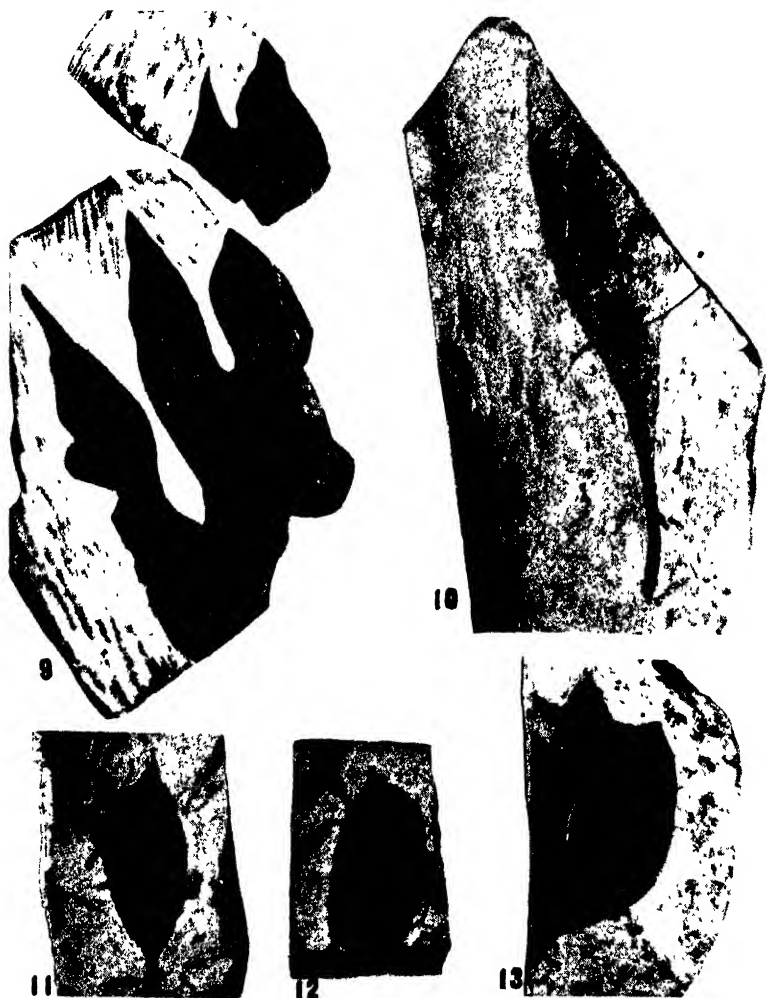
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FIGS. 5-8.—Fig. 5, *Artocarpus lessigniana* (Lesquereux) Knowlton, showing base and venation; fig. 6, *A. lessigniana* (Lesquereux) Knowlton, new form, *dubia* type; fig. 7, *A. lessigniana* (Lesquereux) Knowlton, new variant; fig. 8, *A. lessigniana* (Lesquereux) Knowlton, *dubia* type.

kindly gave the specimens a critical examination and suggested their probable relationship to *Artocarpus*. In the meantime large new col-



FIGS. 9-13.—Fig. 9, *Artocarpus lessigniana* (Lesquereux) Knowlton; fig. 10, *A. lessigniana* (Lesquereux) Knowlton, lower part of new variant showing elongated petiole; fig. 11, *A. lessigniana* (Lesquereux) Knowlton; small leaf of a new variant without terminal lobes showing typical venation; fig. 12, *A. lessigniana* (Lesquereux) Knowlton, another variant; fig. 13, *A. lessigniana* (Lesquereux) Knowlton, relatively broad leaf of new variant.

lections of all obtainable variants had been made from these beds. Among them appeared two huge new forms (figs. 14, 16).

In this great collection are forms which duplicate or at least approximate each of the so-called species, *lessigniana*, *pungens*, *dubia*, *similis*, and *dissecta*. These are shown in the illustrations. The writer is therefore convinced that they are all leaf variations of one and the same species, and that the five "species" here considered should be combined under the name *Artocarpus lessigniana* (Lesquereux) Knowlton, which has the right of priority. The following is a list of the most important variations:

ARTOCARPUS LESSIGNIANA (LESQUEREUX) KNOWLTON

Myrica (?) *lessigniana* Lesquereux, U.S. Geol. and Geog. S., Terr. Bull. Vol. 1, 1875 (1876), p. 386; *idem*, Ann. Rept. 1874 (1876) p. 312.

Myrica (?) *lessigii* Lesquereux, U. S. G. S. Vol. 7 (Tert. Flora), 1878, p. 136, pl. 64, fig. 1.

Artocarpus lessigniana (Lesquereux) Knowlton, Science, Vol. 21, 1893, p. 24; Hollick, Geol. Surv. La., Spcl. Rept. 5, 1899, p. 281, pl. 37; Berry, U. S. G. S. Prof. Pap. 91, 1916, p. 194, pl. 26, fig. 1.

Aralia pungens Lesquereux, U. S. G. S. Vol. 8 (Cret. and Tert. Fl.), 1883, p. 123, pl. 18, figs. 3, 4.

Artocarpus pungens (Lesquereux) Hollick, Geol. Surv. La. Spcl. Rept. 5, 1899, p. 281, pl. 38, figs. 1, 2; Berry, U. S. G. S. Prof. Pap. 91, 1916, p. 195, pl. 25, fig. 1; pl. 27, fig. 1; pl. 29, fig. 1.

Artocarpus dubia Hollick, Geol. Surv. La. Spcl. Rept. 5, 1899, p. 281, pl. 38, fig. 3; Berry, U. S. G. S. Prof. Pap. 91, 1916, p. 196, pl. 29, fig. 2; pl. 113, figs. 1, 2.

Artocarpus similis Knowlton, U. S. G. S. Prof. Pap. 101, 1917, p. 306, pl. 77, pl. 78, figs. 1, 2.

Artocarpus dissecta Knowlton, *idem*, p. 267, pl. 42, fig. 6.

The leaves vary exceedingly in size and form, the largest reaching a length of about 40 cm. with a breadth of 30 cm., the smallest are about 5 cm. long and 2 cm. wide; entire, trilobate, or pinnately one to several-lobed. They may conveniently be described by dividing them into five types as follows:

Type 1.—Large, elliptical, deeply lobate; *Lessigniana-pungens-similis-dissecta* type (figs. 1, 2, 9).

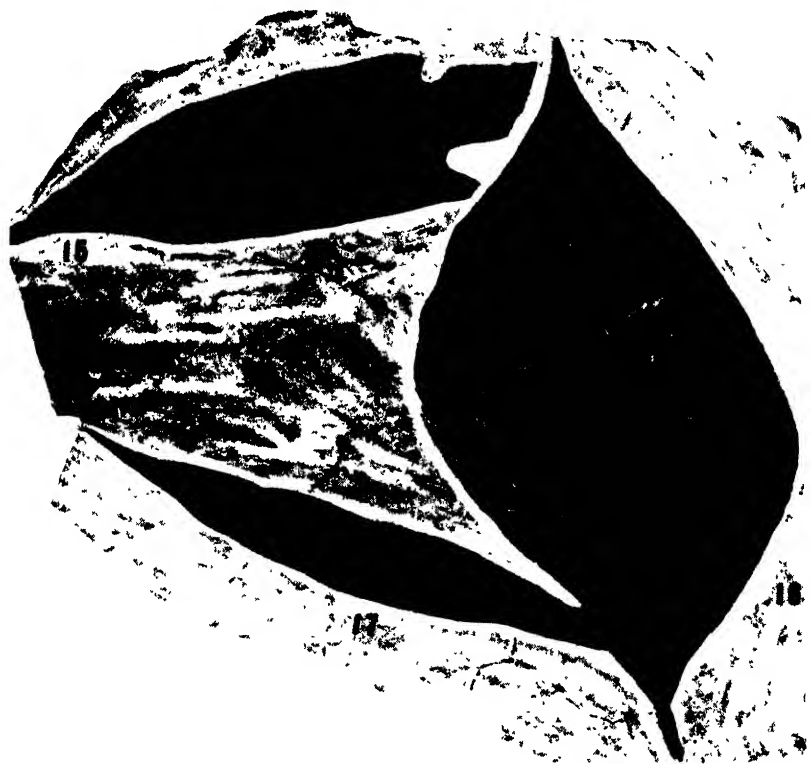


FIG. 14.—*Artocarpus lessigniana* (Lesquereux) Knowlton, large new variant

Type 2.—Large, elliptical, less deeply lobate or nearly entire; new variant (fig. 14).

Type 3.—Large, elliptical or ovate-elliptical, pointed, entire; new variant (fig. 16).

Type 4.—Smaller, more or less slender, oblanceolate to obovate, trilobate at top; in part *dubia* type (figs. 3, 6, 7, 15).



FIGS. 15-17.—Fig. 15, *Artocarpus lessigniana* (Lesquereux) Knowlton, new variant, *dubia* type; fig. 16, *A. lessigniana* (Lesquereux) Knowlton, entire leaf of new variant; fig. 17, *Andromeda* (?) *lanceolata* Knowlton, partly superposed on *A. lessigniana*.

Type 5.—Small, elliptical, ovate or obovate, without lobes; new variants (figs. 4, 11, 12).

In general outline the leaves of the *lessigniana-pungens-similis-dissecta* type are oval or elliptical, widest near the middle, and bear several large and pointed lobes (figs. 1, 2, 9). They are often of great

size, the largest not less than 40 cm. long and perhaps 30 cm. wide from tip to tip of longest lateral lobes, of which there were at least three on a side in the largest specimen. The lobes are typically lanceolate, elongated into a slender and graceful point and are separated by saclike sinuses. The lateral lobes point outward but the distal arch upward. In some specimens the lobes are contracted below, becoming ovate-lanceolate in outline. The midrib is stout and straight; secondaries generally opposite and of two kinds. The larger and longer arise at angles of $40-45^{\circ}$ and pass straight out to the tip of the lobes. The longest of these veins in our specimens is 20 cm., and this lobe, measured from the bottom of the sinus, is 13 cm. long. These secondaries in turn give rise, in the tissue of the lobes, to four or five pairs of tertiaries which are partly craspedodrome, and partly camptodrome in arches along the borders. The secondaries of the other kind are much thinner. Some of these pursue a wandering course outward into the leaf tissues, while others pass out to the fundus of the sinus where they divide and run along one margin of the latter. The nervilles are distinct, percurrent, or form yokes or chevrons between their principals. Not all specimens of this type are large. In the collection from Little Sandy Creek is a small leaf about 8 cm. long and 4 cm. wide, showing one deep sinus on each side.

BERRY (2), revising KNOWLTON and HOLLICK, records similar forms in his work on the Lower Eocene floras. They appear at a number of stations in the Embayment province.

Leaves of the second type, a fine example of which is given in fig. 14, vary from an entire or nearly entire form to one with apical lobes. They may be described as follows: leaves of great size, at least 40 cm. long and 16 cm. wide, elliptical or oblong-oval in outline, widest at the middle. The apex is broadly rounded in general outline, or more or less three-lobed.

Naturally, in such large leaves the skeleton is well developed. The midrib is prominent and perfectly straight, becoming thin in the apical region. The secondaries are pinnate, seven or eight pairs, the lower sub-opposite, the upper widely alternate. The lower pairs arise at angles of about 45° , those of the upper half at 35° . They are prominent and run a nearly straight course, giving rise to several

short tertiaries which pass out to the margin. The nervilles arising from the various members just described are distinct in the region near their principals but become thinner toward the middle of the intercostal areas. A few of them are percurrent but others form chevron-like connections with their opposites. The margins wherever intact are entire. The leaf substance seems to have been coarse and heavy and the surface of some of the leaves was apparently runcinate. The specimen of this type (fig. 14) is by far the largest dicotyledonous leaf observed in the sediments of the Embayment province. The tip has been split for a short distance.

The leaves of type 3 are also large, 20 cm. or more in length and 10 cm. or more in width. Distally the margins incurve to form a narrow and symmetrical acumen. The venation is the same as in the leaves of the other large types. A handsome leaf of this type is shown in fig. 16.

The leaves of type 4 may in turn be divided into two subtypes. Those of the first, which may be called the *dubia* subtype (figs. 3, 6, 8, 15), vary in size and form, the largest being about 15 cm. long and 8 cm. wide, the smaller 5 cm. long and 2 cm. wide, typically three-lobed at the top. The lateral lobes are variable in length, broadly deltoid in form and generally blunt at the apex. The terminal lobe for about two-thirds of its length is broadly spatulate in shape but in the upper third the margins incurve rapidly and are carried upward into an elongated and sharply pointed tip. The fundus of the sinuses is in general smoothly rounded. The margins are entire. The bases are narrowly acuminate, the decurrent margins passing down along the slender petioles, which in some specimens measure 3-4 cm. (fig. 10). The specimens figured should be compared with that of HOLLICK (4, pl. 38, fig. 3).

The venation is clearly marked; the midrib prominent and curving either in its upper or its lower course. The secondaries in the narrow forms are uniformly of one pattern: a pair of nearly opposite, prominent secondaries arise at a distance of 1.5-2 cm. above the base. They diverge from the midrib at an angle of about 30° and curve gently upward, becoming camptodrome along the margins above the middle of the leaf, usually above the level of insertion of the next pair. The latter are also subopposite and diverge in some

specimens at approximately the same angle as the lower, in others at much wider angles. They are straight or slightly curved and pass directly out to the tips of the lateral lobes. In the region between these two-paired laterals, a single unpaired lateral appears in some specimens. Above the upper paired secondaries a pair of short laterals appears in a few specimens, in the region of the terminal lobe; in most specimens, however, these laterals are unpaired and are irregularly spaced.

Leaves of this subtype recall in their shape and in the behavior of the lower lateral primaries those of *Oreodaphne obtusifolia* Berry, but differ in all other respects. There is also a general resemblance, in the larger venation, to *Ficus vaughani* Berry and *Ficus harrisiana* Hollick and to certain species of *Cinnamomum*.

Leaves of the second subtype are abruptly trilobate at the top and are broad in proportion to their length. Some are 9-10 cm. long and 7-8 cm. wide across the top from tip to tip of the lobes, and are of about the same width at or near the middle. In such leaves there may be three or more pairs of secondaries above the long, basal pair. The former diverge in some cases at wide angles, those of the middle region arising almost at right angles to the midrib. From the outer side of the upper secondaries a number of tertiaries arise at wide angles to their principals and pass almost to the border, where they form a series of delicate marginal loops. The finer venation is as described for other types, some of the nervilles being more or less straight and percurrent, while others form yokes or chevrons in the intercostal areas. Leaves of this subtype are shown in figs. 7, 13.

Leaves of type 5 are small and without lobes, or there may be more or less pronounced shoulders at the top or else the halves near the top are markedly unequal. The apex is often extended into a tail-like acumen. These leaves are usually much smaller than those of the other types, although one such specimen is about 12 cm. long and 4 cm. wide. They have narrowly cuneate bases, and a pair of supra-basilar secondaries as in type 4. Their finer venation does not differ in any essential respect from that of the other types. Leaves of this type are shown in figs. 8, 11, 12.

A single highly aberrant form is shown in fig. 4. This leaf is clearly to be allocated to *Artocarpus*, despite its remarkable form.

All of these types have cuneate bases, those of the larger leaves being proportionately wider; the lower margins in all cases are decurrent along the petiole. In the collection from Little Sandy Creek are many fragments of large leaves of which only the basal portions remain. In none of the figures of either *A. pungens* or *A. lessigniana* to which the writer has access is the basal part of the leaf clearly shown. The fragment in fig. 5 is introduced to show the basal region and the remarkably stout midrib of a large leaf.

The petioles of some of the specimens, particularly of the third and fourth types, are elongated, in one specimen reaching a length of 4 cm. or more. In this specimen, shown in fig. 10, the margins are deeply decurrent along the petiole.

The petioles in many specimens are dilated at their bases and usually both petiole and midrib are longitudinally striated, as are frequently the larger secondaries.

All of the specimens described, except the one shown in fig. 1, were recovered from beds which were worked for not more than 15 feet along the outcrop and for a width and depth of not more than 3 feet. In this limited area countless numbers of more or less complete leaves were found, together with great quantities of other leaves. The beds represent muds which in early Eocene times were deposited in the bottom of a shallow coastal lagoon, the shores of which were covered with a rank vegetation. A prominent member of this association was *Artocarpus*. Leaves of this plant, of all the types here described, occur intermingled in these beds, often compacted together. The lobate leaf shown in fig. 9 lay about one inch directly beneath one that was almost a duplicate of the nearly entire leaf shown in fig. 14.

The records show in many cases that *A. pungens* and *A. lessigniana* occur together in beds of the Embayment province and in those of the Rocky Mountain region. It does not seem probable that two or more species of *Artocarpus* lived beside each other in those old swamps. They do not so live today. In short, I am convinced that all the leaves described in this paper are merely leaf variations

of one and the same species. Table I shows the distribution of the various "species" of *Artocarpus*.

Inspection of table I shows that at Couchatta, Louisiana, *lessigniana*, *pungens*, and *dubia* all occur in the same beds; *pungens* and *dubia* appear in the De Soto beds, while at Naborton, *pungens* is found in beds not far from those containing *dubia*.

TABLE I

	WILCOX FORMATION AT						LARAMIE IN COLO.	DENVER IN COLO.	RATON IN COLO.	VERMEJO IN COLO.
	Couchatta, La.	Naborton, La.	De Soto, La.	Shreveport, La.	Grenada, Miss.	Benton, Ark.				
<i>A. lessigniana</i>	X						X			
<i>A. pungens</i>	X	?	X		X	X		X		
<i>A. dubia</i>	X	X	X	X						
<i>A. similis</i>									X	
<i>A. dissecta</i>										X

Occurrence: Laramie formation, Colo.; Vermejo formation, Colo.; Denver formation, Golden, Colo.; Raton formation, Colo.; Wilcox formation, Ark., Miss., La.; Indio formation, Barton's ranch and Little Sandy Creek, Bastrop County, Texas. Collected from the latter by O. M. BALL. All specimens figured, except no. 1, are in the collection of the A. & M. College of Texas.

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THE OZARK WHITE CEDAR

JOHN T. BUCHHOLZ

(WITH TWO FIGURES)

In the Ozark region in the basin of the White River is a species of *Juniperus* related taxonomically to *J. monosperma* (Engelm.) Sargent, but with a number of rather constant differences which have justified the conclusion that it represents a distinct species. I am naming it *Juniperus ashei* in recognition of W. W. ASHE of the United States Forest Service, who first brought the species to my attention from material collected at Sylamore, Arkansas, in the spring of 1924, at that time pointing out some of the distinguishing differences in the female flowers and seeds. Soon after this I found this species more than a hundred miles farther west, near Eureka Springs, Arkansas, at several points along the White River bluffs, and observed it frequently in the field until the summer of 1926.

While the Arkansas white cedar is related to *J. monosperma*, it is widely separated from the known range of the latter and seems to differ from it in as many features as *J. monosperma* differs from *J. occidentalis* or from some of the other one-seeded junipers. The Arkansas tree may be distinguished from *J. monosperma* by having tetragonal and more slender branchlets, by the color of the gray twigs, by its larger fruits and seeds, by the absence of flattened seeds when the seeds are single, by the shallower furrows on the seeds, by the absence of ridges and by the more pointed tip of the seed; also by the difference at the time of pollination in the female flower from the flowers of *J. monosperma* and by the larger number of sporophylls (stamens) in the male cones as these are given in current descriptions. The female flowers of the Ozark species are not spreading, as may be seen from fig. 1, while those of *J. monosperma* are usually described as spreading.

If the mature branchlets of the flower or fruit-bearing twigs are compared, those of *J. monosperma* usually average between 1.2 and 1.4 mm. in width when the leaves are opposite in pairs, and between

1.3 and 1.6 mm. in width when the leaves are in threes; while those of the Arkansas tree average 0.7–1.1 mm. in width when the leaves are in pairs and 0.9–1.2 mm. when the leaves are in threes. Fig. 2 shows shadow prints made with parallel light rays and is therefore accurate in regard to dimensions.

The twigs of *J. monosperma* have a light reddish or copper-colored bark, while the bark of the twigs of the Ozark tree is dark gray, be-

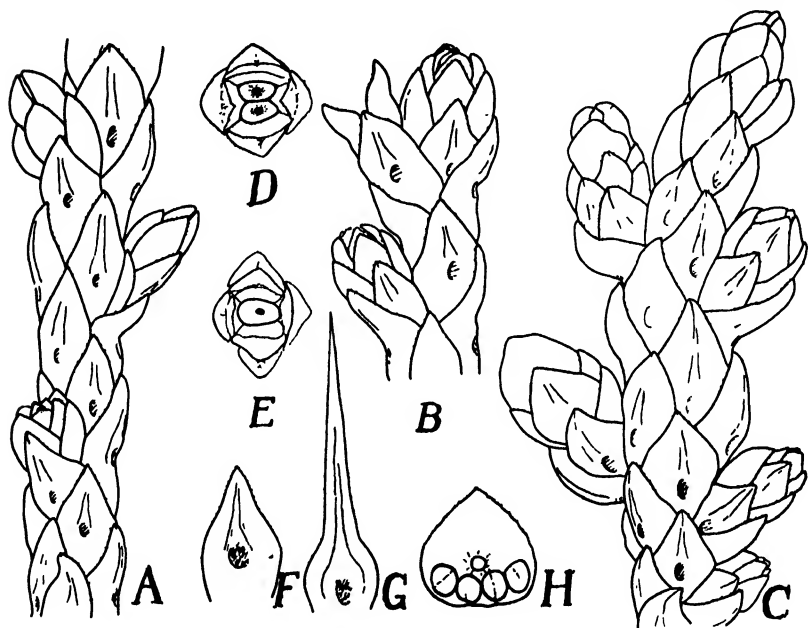


FIG. 1.—Twigs and flowers of *Juniperus ashei*: A, B, branches with leaves in pairs, A bearing two unopened female cone buds and one open cone at time of pollination; C, twig with leaves in threes (6-ranked) bearing flowering and unopened female cones; D, E, cones of branch B as viewed from above, showing cones with two ovules and single ovule; F, average leaf from mature branchlet; G, same from foliage near tip of vigorous vegetative shoots and juvenile foliage; H, microsporophyll (stamen) showing four sporangia; $\times 12$.

coming ashen gray with age. In the smaller twigs which are still clothed with the armor of dead leaves the color of the leaves is yellowish in *J. monosperma*, and dark gray, black, or brown in *J. ashei*, resembling twigs of *J. virginiana* more nearly than those of *J. monosperma*.

The seeds of *J. monosperma* average 3.5 mm. wide and 4.5 mm. long, are 2-3-ridged, slightly obtuse, sometimes flattened, especially toward the apex, and distinctly furrowed or grooved, while those of the Ozark white cedar average 4 mm. wide and 5.4 mm. long, are

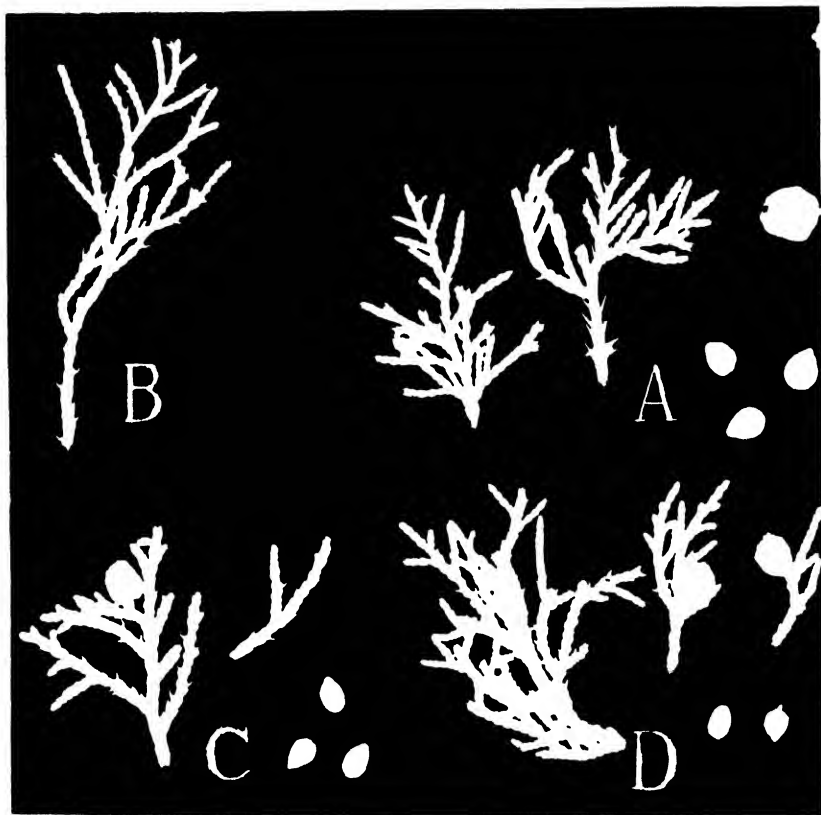


FIG. 2.—Photographic shadow print showing actual dimensions of *Juniperus ashei*: A, from Eureka Springs, Arkansas and B, from Sylamore, Arkansas, together with fruit and seeds; C, similar shadow print of fragment of *Engelmann* type specimen of *J. monosperma*, from Colorado and D, from collection at Pike's Peak, National Forest, showing smaller sized fruit and seeds and stouter twigs.

pointed and not flattened, without angles or ridges, without grooves or having only very shallow grooves, and with a relatively small hilum.

Juniperus ashei occurs in association with *J. virginiana* and might upon superficial examination be confused with this species, but *J. ashei* has a different habit of growth, its stem usually forking at or near the base, while *J. virginiana* is invariably single stemmed; and in general *J. ashei* has a one-seeded fruit. When fruits are not available as a means of separating these species, as in the case of seedlings, small plants, and male trees, it has been found practicable in field work to separate them by examining the exposed margin of the leaves, using for this purpose a 15× or 20× hand lens with the leaves held against the light. The finely serrate or serrate-dentate margin of the leaves of *J. ashei* at once separates it from *J. virginiana*, which has entire leaves. *J. ashei* may be distinguished from *J. virginiana* also by its stiffer branchlets and by the more pleasant odor of the crushed twigs.

My former colleagues who have been observing these species have noted recently that the staminate cone scales of the white cedar are tipped with brown when in their prime while those of the red cedar are yellow. In localities where the *Gymnosporangium* rust galls are abundant the white cedar is easily distinguished since it is immune to this rust. Many farmers living in localities where *J. ashei* is somewhat abundant have long distinguished it from the other species, and apply to it the name "white cedar" on account of the thicker white sapwood and the pale brown color of the heartwood. The wood is highly prized for posts and like uses on account of its durability in contact with the soil.

***Juniperus ashei* sp. nov.**—Arbor dioica, submediocris, fruticiforme ramosa; foliis oppositis ternisve, minutissime denticulatis serrulatisve, glandulosis vel eglandulosis, appressis, acute ovatis, 1-1.8 × 0.5-0.9 mm., in ramulis juvenilibus acuminatis subulatisve, 2-8 mm. longis, cinereis sed non glaucescentibus; staminibus circ. 12, imbricatis, scariosis, obtusis, denticulatis erosive, basi 3-5-sporangiiferis; ovulis 1-2, erectis; fructibus luridis, globoso-ovatis, 7.85 × 6-7.5 mm.; seminibus solitariis binisve, late ovatis, 3.5-4.5 × 5-5.8 mm., acutatis, levissime sulcatis sed non angulatis.

Leaves in pairs or less frequently in threes, minutely serrate or serrate-dentate on the margins and with or without a large oval

gland on the back, dark gray-green but not glaucescent; on mature fruiting or flowering branches closely appressed, ovate, acute, 1-1.8 mm. long and about 0.5-0.9 mm. wide; leaves on seedlings or on the tips of vigorous branches 2-8 mm. long, spreading and acuminate or awl-shaped, the foliage of young plants yellowish with a light brown color on the twigs where exposed. The foliage of young plants and terminal twigs is yellowish-green to light brown, the mature branches a darker green, and the old leaves form a dark armor clothing the smaller twigs, becoming chocolate-brown upon drying, and finally black. As the black leaf armor falls off, a gray bark is exposed on the twigs which becomes ashen gray with age. Mature flowering or fruiting branches usually have ultimate segments 0.7-1.1 mm. in width when leaves are in pairs and 0.9-1.2 mm. when leaves are in threes. The internodal distance of the mature branches usually measures 0.55-0.85 mm. (average 0.7 mm.).

Flowers appearing about the middle of March, dioecious, the staminate forming terminal cones of about 12 overlapping peltate sporophylls (stamens), these scarious, rounded or obtuse, and erose or dentate on the tip and margins, bearing 3-5 sporangia at the base; pistillate flowers representing terminal cones or borne on short spur branches, the flowers no wider than the branches and not spreading at the time of pollination, consisting of one (or two) erect, ovate, flattened, brown or tan ovules, tipped with a red micropyle, surrounded by 3 pairs of overlapping cone scales, these surrounded by the upper leaves of the branch. Cone scales enlarging slightly about a week or ten days after pollination, remaining inactive for a period, then enlarging rapidly as they become fleshy in fruit. Fruit glaucous, dark blue, with a thin sweet flesh, globose-ovoid, 7-8.5 mm. long, 6-7.5 mm. wide, with one or sometimes 2 seeds. Seeds broadly ovate, not flattened when single, 3.5-4.5 mm. wide and 5-5.8 mm. long, pointed, without ridges or angles, and with several very shallow grooves extending lengthwise.

A tree of shrubby aspect becoming 12-20 feet high, usually with several trunks coming from a short, fluted main trunk or sometimes branching at the ground, forming a broad, globular or open, irregular crown. Bark thin, ashen gray in long, narrow, persistent, shreddy

scales. Wood heavy, with pale brown heartwood and a broad zone of nearly white sapwood.

Distribution.—Basin of the White River from the region southwest of Eureka Springs, Arkansas, and along the course of the river through southern Missouri and southeastward to the region near Sylamore, Arkansas, occupying a belt about 120 miles in length and 40 miles in width within which it occurs only upon suitable soils and sites. It occurs in association with *J. virginiana* but is more limited in its distribution, being confined to the zone of the dolomitic and calcareous outcrops and bluffs of the White River basin in the western part of its range, and apparently associated with sandstone outcrops and bluffs in the eastern part of its range.

Material examined.—ARKANSAS (*Juniperus ashei*): Sylamore, W. W. Ashe, Jan. 9, 1923, Apr. 25, 1924, and Apr. 28, 1925, Sept. 16, 1923, and Mar. 6, 1924, male- and female-type specimen, in herb. W. W. Ashe (cotype material on deposit at Herb. Mo. Bot. Gard. and elsewhere); Mountain Home, *idem*, April, 1926 and March, 1929, Herb. W. W. Ashe; Eureka Springs near White River bridge and Beaver Post Office, J. T. Buchholz, in 1924, 1925, 1926, Herb. University of Arkansas; Beaver, E. J. Palmer 29318, Oct. 23, 1925, *et idem* 31428, Jul. 26, 1926, Herb. Arnold Arboretum; *idem* 29820 *et* 29822, Apr. 29, 1926, Herb. Univ. Arkansas; D. Demare 6356 *et* 6357, Mar. 23, 1929, Herb. Univ. Arkansas; Monte Ne, D. M. Moore 779, Mar. 29, 1929, *et idem* 813, "on cliffs of White River," October, 1928, Herb. Univ. Arkansas.

MISSOURI (*Juniperus ashei*): Eagle Rock, B. F. Bush 3153, Aug. 8, 1905 (probably this species; Herb. Arnold Arboretum); "bald knob" along Arkansas-Missouri border, on dolomite slopes, E. J. Palmer 31440, Jul. 27, 1927, Herb. Arnold Arboretum, *et idem* 29844, Apr. 30, 1926, Herb. Univ. Arkansas.

COLORADO (*Juniperus monosperma* (Engelm.) Sarg.): "On limestone hills," Canyon City, Geo. Engelmann, Sept. 26, 1874, apparently the type specimen bearing original label *J. occidentalis* Hook. and annotation "Seminibus saepe protrusis," Herb. Mo. Bot. Gard.; La Veta, P. A. Rydberg and F. K. Vreeland 6587, Herb. N. Y. Bot. Gard.; Pike's Peak Nat. Forest, Rees Philips 21 (seeds only) and 19. twigs, seeds, male cones, 1924, Herb. W. W. Ashe; Trinidad, *anon.*,

seeds larger than type material but slightly flattened, Herb. U. S. Nat. Mus. (sheet no. 760586).

ARIZONA (*Juniperus monosperma*): Flagstaff, Fort Valley Exp. Station, G. A. Pearson, Febr. 19, 1924, seeds small, angled or flattened near tip and grooved, mostly exposed in fruit, Herb. U. S. Nat. Mus.; Coconino National Forest, *idem* 2873, Sept. 6, seeds small, flattened, grooved, Herb. U. S. Nat. Mus.; San Francisco Mts., Knowlton, twigs stout, seeds small, grooved, flattened toward tip, Herb. U. S. Nat. Mus.; Adamona, Griffiths, Aug. 10, seeds large, angled and grooved with large hilum, Herb. W. W. Ashe.

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BRIEFER ARTICLES

OXYQUINOLINE SULPHATE AS A PRESERVATIVE FOR PLANT TISSUES

Although the botanist who collects material for anatomical or taxonomic examination has many suitable preserving agents at his command so long as he remains close to the laboratory, in the field he has to consider seriously the problem of preservatives. Alcohol and formalin have usually been employed for such work, but however well adapted these are for the actual preserving, they offer serious disadvantages, not only in transporting the solutions to the point where they are to be used, but also in bringing the preserved material back to the laboratory. In addition to loss by leakage or evaporation and other difficulties, customs regulations are likely to prove troublesome, especially with alcohol.

The 1928 HUMBERT-SWINGLE Madagascar plant exploration trip¹ tried out water-soluble oxyquinoline sulphate as a partial substitute for the more bulky formalin and alcohol, and much more satisfactory results were obtained from a single ounce of oxyquinoline sulphate powder than from the gallons of alcohol and formalin employed.

Oxyquinoline sulphate has long been known to the medical profession as a practically non-poisonous, non-corrosive disinfectant, with relatively low germicidal action but with high antiseptic or preservative properties. It was originally introduced under the trade name Chinosol, supposedly as the potassium salt of oxyquinoline sulphonie acid; apparently, however, the effective antiseptic agent is simply the oxyquinoline sulphate.² The product seems to be better known to the zoölogist than to the botanist,³ but it has been used in at least one botanical laboratory, the Institut für Allgemeine Botanik, Hamburg.

The use of oxyquinoline sulphate in preserving botanical specimens is simplicity itself: the material is merely put in the container, covered with water, and a little powder added, guessing at the amount. Any

¹ Nat. Geog. Mag. August, 1929.

² Jour. Amer. Med. Assn. 50:293. 1908; Ann. Rpt. Chem. Lab. Amer. Med. Assn. 12:87. 1919.

³ Gross, Centralbt. Bakt. I. 111:315. 1929.

amount, from 1 per cent or stronger to 0.1 per cent solution or even weaker, is satisfactory for stopping bacterial action with ordinary plant material. The material has no special penetrative power, hence the larger the specimens, the more powder should be used. Solution lost by leakage apparently has no disagreeable staining or corrosive action; unless some evidence of bacterial activity is shown, it is safe merely to replace small amounts of lost solution with water.

Oxyquinoline sulphate solution has no drying and shrinking effect upon plant tissues, nor does it evaporate like alcohol or formalin; material can be taken from the solution, handled in the air for hours, and then replaced. Sections can be cut and handled from the solution just as from water, or the plant material can be run up through the alcohol series. Although no oil-immersion studies were made, all material examined (for example, latex cells of *Euphorbia*) seemed to be perfectly killed and fixed, and there was no indication of any undesirable precipitates in the tissues.

Apparently the only serious drawback of this solution as a plant preservative is the fact that it forms a precipitate with iron, hence the advisability of using glass or porcelain containers. Possibly also this property would make the use of Haidenhain's haematoxylin inadvisable.

Perhaps subsequent use in the botanical museum will reveal some points whereby oxyquinoline sulphate is inferior to alcohol and formalin as a permanent preserving fluid; but the gratifying results which it has given so far recommend it as the best preservative for the use of the botanist in the field.—CHARLES F. SWINGLE, *United States Department of Agriculture, Washington, D.C.*

CURRENT LITERATURE

BOOK REVIEWS

Plant geography of Balkan Peninsula

A recent volume by TURRILL¹ is the first of what will probably become a series of major contributions to the Oxford Memoirs on plant geography. This book is unique in the order and detail of its presentation, and sets a high standard for succeeding volumes on plant geography of special regions.

The volume should attract considerable attention because of human interest in the region and its consideration of the effects of human activity upon the vegetation. It has a certain formidableness which will preclude casual reading, but the very features that make the book imposing tend toward increased value and facility for reference purposes. Particularly to be recommended in this respect and as conserving space is the practice of assembling the data in tables and by symbols. In chapter XII, for example, the floristic and phytogeographic data are summarized for the families of the Balkan Peninsula phanerogams in the space of 58 pages by the use of symbols, which information would otherwise fill a small book.

There are adequate chapters on the geography, geology, soils, and climate of the region. From the ecological point of view there are chapters on duration and life forms, flowering periods, habitat classification, altitudinal zonation, plant communities, and plant succession. From the floristic point of view there are detailed considerations of dispersal, distributions within the Peninsula, distributions outside the Peninsula, and the interesting problems presented by endemic and relic species. All this is more or less concluded by application and criticism of WILLIS' age-and-area hypothesis.

TURRILL's floristic treatment of this rich region, with 6530 accepted species, brings out the relations of the flora to neighboring regions in Asia Minor, Asia, Africa, and Europe. He recognizes two main types of flora and vegetation in the Balkan Peninsula, the Mediterranean and the Central European. The former have the predominating species xeromorphic in structure or with a life history otherwise adapted to surviving the summer drought; the Central European species are essentially composed of mesophytic species whose life history has its resting phase in winter. Climate is the most important factor in the distribution of the different vegetation communities, but it is stated that it is difficult to overemphasize the modifying influence of man.

¹ TURRILL, W. B., *The plant-life of the Balkan Peninsula: A phytogeographical study*. 8vo. pp. xxii+490. *pls. 10. 11 maps*. Oxford: Clarendon Press. 1929. \$10.

As TANSLEY states in the preface, "we have nothing in England to place beside the stately series published in Leipzig under the general title *Die Vegetation der Erde*, but this first volume of the Oxford Memoirs on plant geography constitutes a beginning that would be extremely difficult to excel."—S. A. CAIN.

Genic analysis

Geneticists may well be grateful to MATSUURA for his recent monograph.² Laboriously he has collected all the significant information published between 1900 and 1925 inclusive upon the identification of genes in seed plants and the establishment of their linkage relationships. Genera are arranged alphabetically. Under each genus, the genes are grouped on the basis of the part of the plant body affected or the type of character produced. The effects of each gene are described clearly and complete references made to the original literature.

Four indexes are provided: author, subject, species, and family. The bibliography includes 1341 titles, representing the work of over 500 investigators. Each title is followed by a brief description of the content of the paper.

This volume will prove a tremendous convenience to teachers of genetics and those engaged in or directing genetical investigation with plants. It is a satisfaction that the author promises to produce a companion volume bringing genic analysis in phanerogams up to date and including data upon cryptogams.—M. C. COULTER.

Exploring for plants

In his world-wide search for plants that might prove of economic importance to the United States, FAIRCHILD³ visited lands little known to most botanists. His experiences, charmingly told, contain a store of information concerning unknown plants, many facts about the food and clothing used in other lands, and countless amusing and entertaining incidents of travel. Among the regions visited were Northern Africa, the Canary and Balearic Islands, Ceylon, Sumatra, Java, and long stretches of the west coast of Africa.

The book is written in an easy, readable style, and the reputation of the author is sufficient guarantee for its scientific accuracy. It contains information for both the scientist and the layman; it is well illustrated; and it seems safe to predict that its success will far surpass the modest ambition expressed by the author: "I shall be content if a perusal of its pages shall convert a single person to the romantic life a deep study of plants can give."—G. D. FULLER.

² MATSUURA, H., A bibliographical monograph on plant genetics (genic analysis). 1900-1925. pp. 499. Tokyo (New York Agent, Henry George Fieller). 1929.

³ FAIRCHILD, D., Exploring for plants. From notes of the Allison Vincent Armour Expeditions for the U.S. Department of Agriculture, 1925, 1926, and 1927. pp. xx+591. figs. 180. New York: Macmillan Co. 1930. \$5.

THE BOTANICAL GAZETTE

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UNROLLING OF LEAVES OF *MUSA SAPIENTUM* AND SOME RELATED PLANTS AND THEIR REAC- TIONS TO ENVIRONMENTAL ARIDITY¹

ALEXANDER F. SKUTCH

(WITH TWENTY-THREE FIGURES)

Introduction

In two previous publications the anatomy (5) and the development and morphology (6) of the leaf of the banana have been discussed. The present paper is devoted to a description of the unfurling of the lamina, and of the development of the tissues of the pulvinar band, by the agency of which the lamina halves fold together beneath the midrib in the middle of dry days and spread out again toward evening. For purposes of comparison, I shall discuss the same points in several other genera, representing each of the four families into which the Scitamineae is divided.

The first paper figured and described a peculiar hypertrophy of the cells of the upper water tissue at points where the longitudinal furrows in the surface of the lamina (caused by pressure exerted upon it while still rolled within the false stem) intersect one of the principal veins. At that time I was unable to explain the significance of the enormous anticlinal elongation of these cells, but merely recorded the anatomical peculiarity. During a recent visit to Panama experiments were performed on the mechanism of the unrolling of the lamina which make it clear that hypertrophy of the cells of the water

¹ Botanical contribution from the Johns Hopkins University, no. 109.

tissue at the points in question is the direct result of the difficulty in flattening out these folds when the leaf unrolls.

Since my earlier description of the banana leaf was written, Löv (3) has studied the mechanism of the unfolding of monocotyledonous leaves. In most families of this class the leaf, whether plicate, convolute, involute, or otherwise disposed in vernation, does not expand as a result of the properly modulated or harmonious growth of all of its tissues, but rather through the rapid enlargement of certain more or less specialized cells which up to the time of unfolding have lagged behind the others in development. These cells, which are designated "unfolding cells" (Entfaltungszellen) in the German literature on the subject, and for which I propose the term *expansion cells*, from their rôle in the expansion of the lamina, are of necessity situated on that surface of the leaf which is concave in vernation. In most monocotyledons this appears to be the upper or ventral surface, although in the plicate leaves of palms expansion cells are found alternately on the dorsal and ventral surfaces, as governed by the direction of the folds. Expansion cells may be either epidermal, hypodermal, or more deeply situated. In the Gramineae, Cyperaceae, Juncaceae, Liliaceae, Amaryllidaceae, and Commelinaceae the information at present available indicates that the expansion cells are typically epidermal, although in many cases they are aided by more deeply situated tissues. In the Pandanaceae, Palmae, and Scitamineae the expansion cells are almost always hypodermal (water tissue). The epidermis contributes at most a very subordinate aid in the process of unfolding, but the mesophyll (as distinct from the hypoderm) may in some cases be of substantial help. In the Orchidaceae there is considerable variation in the position of the expansion cells. In the whole order of the Helobiae they are apparently absent, as they are from the Bromeliaceae and the leaves of the Araceae (although present in the spathes of certain species) and from a series of genera in the Amaryllidaceae. In these leaves the unfolding is accomplished by the coordinate development of all of the tissues, the progressively increasing growth of all cells from the lower surface to the upper.

In this rapid survey it has been necessary to confine statements to the broadest generalizations; the numerous exceptions may be found

in Löv's memoir. In some species great variation is found even between different regions of the same leaf. Thus in the upper portion of the leaf of *Homeria collina* the expansion cells are developed from the hypoderm and the mesophyll, while in the basal portion the epidermis also contributes to their number.

In regard to their distribution over the surface of the leaf, considerable variation exists. In the Commelinaceae, for example, the epidermis over the whole of the upper surface consists of expansion cells. In many grasses, palms, and other leaves with particular lines of folding, the expansion cells are more or less restricted to definite longitudinal series at these places.

Microscopically expansion cells are characterized by their thin, generally unsuberized walls and large clear lumina. Tannin, crystals, oil bodies, and leucoplasts are seldom present. Chlorophyll is present only in expansion cells which develop from the mesophyll, and even in these it typically occurs only in small amounts.

The expansion cells of the grasses, which generally contrast sharply with the neighboring epidermal cells, were apparently the first to receive the attention of botanists, and were described by DUVAL-JOUVE (1) in 1875. Later TSCHIRCH (7) devoted a special study to the inrolling of the leaves of xerophytic grasses, which is at least in part effected by their expansion cells. The expansion cells of grasses are apparently the only ones mentioned in the text-books, and then only in regard to the opening and closing of these leaves in response to wetting and drying, rather than in relation to their primary function in the unfolding of the young leaf. RUDOLPH (4) has described the mechanism of unfolding of the leaves of palms.

Unrolling of banana leaf²

At the moment the tip of the young leaf emerges from the top of the false stem, the lamina is practically full-grown. After tearing away the leaf sheaths, the tightly rolled lamina may be unfurled without

² The descriptions and drawings refer to apparently undescribed varieties of the subspecies *seminifera* of *M. sapientum* introduced to Almirante, Panama, from the Philippine Islands under various native names, but agreeing so closely in vegetative characters as to be indistinguishable from one another. The varieties of the banana are legion, and their names even more so, but there seems to be essential agreement within the species in anatomical characters, and the present account gives a description of the typical common cultivated varieties.

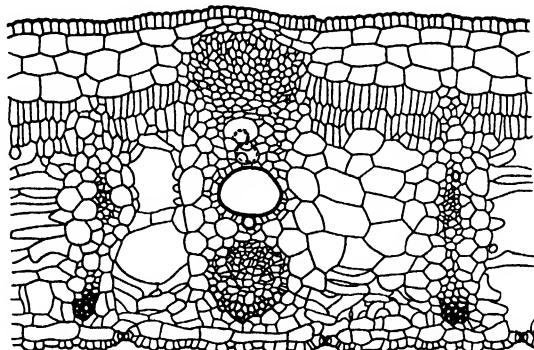
difficulty. The tissues, except perhaps at the extreme tip, are still ivory white, and the upper surface of the two sides of the lamina is perfectly smooth, without a trace of the transverse ribs so conspicu-



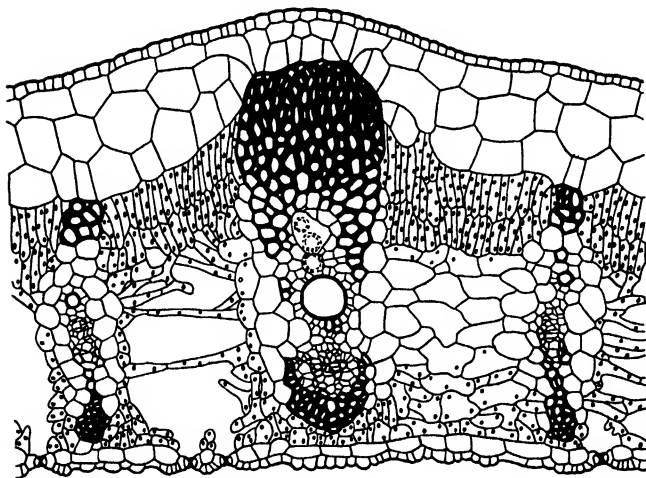
FIG. 1.—Partially expanded lamina of young banana plant (note prominent transverse ribs and reflexed position of sides of lamina at apex).

ous in the mature leaf (fig. 1). These ribs are not primarily places of greater thickness in the lamina, but merely corrugations, the result of the upward curvature of the tissues on either side of the principal veins, so that the latter lie above the general surface of the leaf. On the lower side each ridge is represented by a furrow. The ribs are

formed as the leaf unrolls. They become apparent first at the apex of the left half of the lamina, which is always outermost in the convolute vernation. From this point their formation proceeds basally



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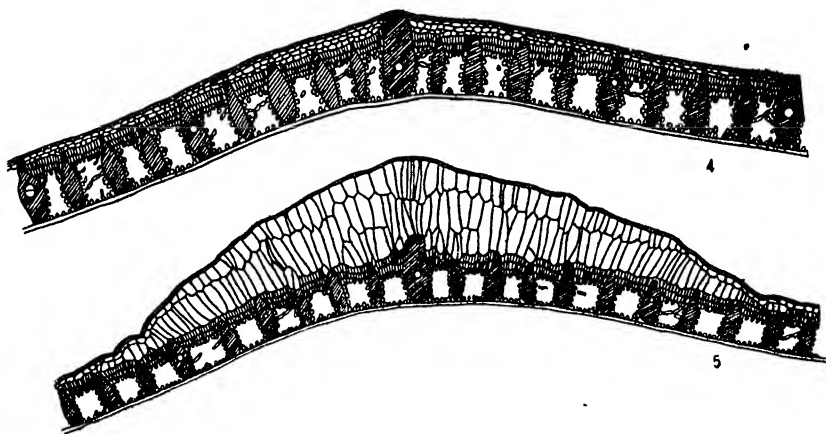


3

FIGS. 2, 3. —Fig. 2, transverse section through principal and two subordinate veins of lamina not yet unrolling; fig. 3, same, from mature lamina; $\times 135$.

and to the right, keeping pace with the emergence of the leaf, which is effected by rapid elongation of the sheath, particularly its basal portion. Thus the regions of the lamina which are the first physically able to unroll are the first to show the formation of ribs.

Figs. 2 and 3 illustrate the alterations which occur in the cells themselves. Fig. 2 shows a cross-section through principal and subordinate veins of a large leaf just appearing above the false stem, but from near the center of the length of that leaf, and hence from tissues still far down the false stem. Fig. 3 was drawn by camera lucida on the same scale as the preceding, and gives the appearance of the corresponding portion of a mature expanded leaf. Certain evident changes have taken place during the interval between the stages represented by the two drawings, the most conspicuous of which are: (1) the



FIGS. 4, 5.—Fig. 4, transverse section through principal and neighboring subordinate veins of normally expanded lamina; fig. 5, same, from lamina bound 10 days; semi-diagrammatic, water tissue drawn with camera lucida; vascular bundles shaded; $\times 24$.

great enlargement of the cells of the water tissue lying above and on either side of the principal vein; (2) the anticlinal elongation and thickening of the walls of the fibers on the upper side of the principal vein and the thickening of those above the subordinate veins. These cross-sections do not extend far enough laterally to show that the principal vein has been raised above the general surface of the lamina (fig. 4). The changes on the lower side are much less conspicuous. The fibers already show considerable thickening in fig. 2, while those above the xylem are extremely thin walled. The palisade cells have experienced a certain amount of elongation, and the upper epidermal cells have enlarged considerably, but both changes are overshadowed by those of the upper fibrous bundle and the water tissue. It is

evident that the upper portion of the leaf lags behind the lower in its rapidity of differentiation until the moment of unrolling, at which time its development, in relation to a particular function, is greatly accelerated.

Lignification of the fibers lying on the lower side of the vascular bundles of the lamina proceeds *pari passu* with the unfurling of the lamina. The first trace of lignification is evident in the upper left corner shortly before the coils begin to loosen. Thence it proceeds basally and to the right, and lignification of any particular portion of the lamina has usually at least begun by the time that portion begins to unfurl. The production of chlorophyll spreads over the leaf in the same manner, usually keeping a step ahead of the former process, and suggests that the incidence of light is responsible for the initiation of lignification. The same is true in regard to lignification of the hypodermal cells beneath the outer surface of the sheaths (5). The walls of the fibers above the bundles, which never become lignified but at maturity give the reactions of suberized membranes, are at most very slightly thickened during the process of unfurling, and gradually increase in thickness after the leaf has expanded, until finally they are as thick as those of the dorsally situated fibers.

The great enlargement of the turgid cells of the upper water tissue above and particularly on either side of the principal veins results in pushing these veins above the general surface of the leaf. Since the upper surface is concave in veneration they are forced inward, and occupy, throughout the helix, a curvature of smaller radius than immediately adjacent tissues of the lamina (fig. 6); hence the whole principal vein is thrown into a state of compression in relation to the subordinate veins and the intermediate tissue. The turgid cells of the upper water tissue are most affected by this change, and eventu-

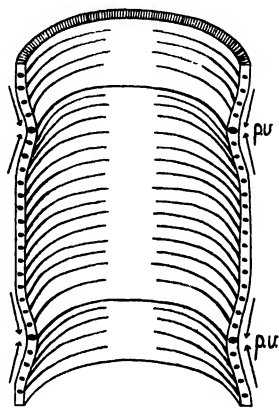
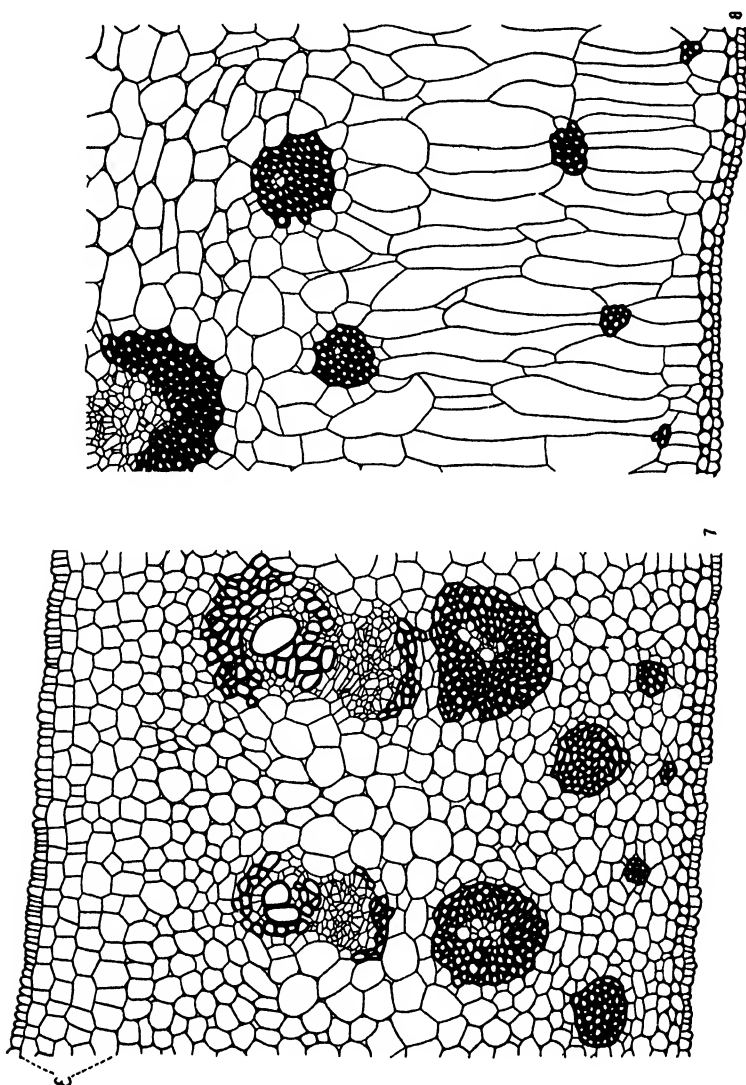


FIG. 6.—Illustrating method of leaf unrolling; arrows show direction of forces resulting from enlargement of expansion cells; *pv*, principal vein.

ally occupy a position enabling them to exert their expansive force to the greatest mechanical advantage. The lower side of the leaf,

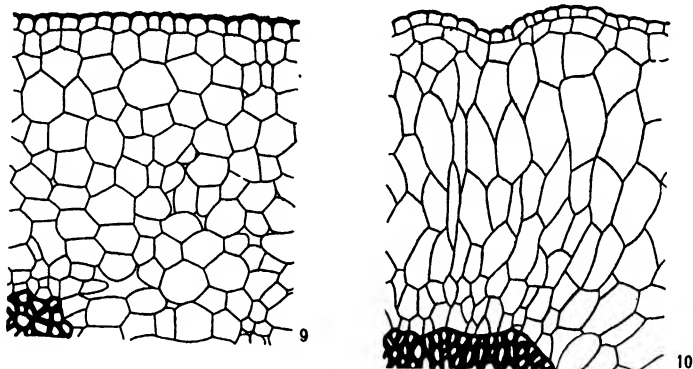


FIGS. 7, 8.—Fig. 7, portion of cross-section of pulvinar band of leaf just appearing from false stem: *e*, expansion cells; fig. 8, lower portion of cross-section of pulvinar band of mature leaf, showing prismatic cells; $\times 112$.

which contains fibers already showing lignification, is relatively resistant to stretching, while the entire upper portion, the fibers in which are still thin walled, is much more easily stretched. The diver-

gent behavior of these two sets of fibers is accordingly of great importance in the unfurling of the leaf.

Meanwhile certain changes are occurring in the tissues lying immediately adjacent to the midrib, which have been called the pulvinar bands (5). These bands differ from the remainder of the lamina, among other things, by the absence of lacunae. Were the two lamina halves merely to flatten out without the help of the pulvinar bands they would stand above the midrib with their upper faces together and parallel (5, fig. 8). It is the primary function of the pulvinar bands to bend out the lamina halves so that they come to lie



FIGS. 9, 10.—Fig. 9, upper portion of cross-section of pulvinar band of normally unfurled leaf, showing expansion cells (cf. fig. 7e); fig. 10, same, from leaf bound 23 days, showing hypertrophy of expansion cells; $\times 112$.

in a plane. Fig. 7 represents a section through the pulvinar band of a large leaf shortly before unfurling. The section was cut transverse to the midrib and parallel to the veins of the lamina. The fibers have already become thick walled (here they rarely become lignified). The cells of the upper water tissue, 4-5 layers deep, are still immature, and as the leaf unfurls they enlarge to the proportions shown in fig. 9, a section through the corresponding region of a mature expanded leaf. By their great enlargement and turgescence these cells bend out the lamina halves along the sides of the midrib, until they actually overstep the degree of flexion necessary to flatten out the leaf and are inclined backward (fig. 1, upper half of unfurling leaf). The process by which they are brought back to position is discussed in a later section of this paper.

EFFECT OF PREVENTING UNROLLING

By binding the apex of the leaf with soft twine when it first appears, and then continuing to wrap the twine basally as successive portions emerge, over a period of several days, it is possible to prevent unrolling of the lamina without injuring it. By this interference all of the growth processes which effect expansion of the leaf are greatly exaggerated.

By leaving the leaf bound for a week after it has completely

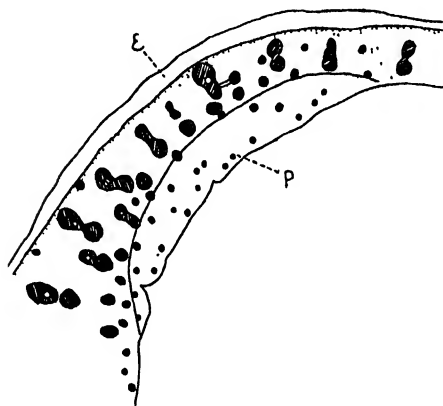


FIG. 11.—Diagrammatic cross-section through pulvinar band of normally expanded leaf, showing expansion cells (*e*) and prismatic cells (*p*); fibrovascular bundles shaded; $\times 11$.

emerged, striking results are obtained. When the cord is removed the left half slowly but visibly unrolls, and then begins to roll inward from the margin with the lower side concave (fig. 12), in just the opposite direction from vernation. The principal veins are marked by great welts which are lighter in color than the remainder of the leaf and are hard and turgid to the touch. The strongest of the subordinate veins, one of which lies between each pair of principal

veins, are also sometimes swollen, but always to a much less degree, and not continuously. The leaf is much more strongly ribbed than normally. Microscopical examination reveals that the swollen appearance of the principal veins is caused by the great hypertrophy³ of the cells of the upper water tissue, which elongate anticlinally, the only way they are free to grow. This hypertrophy extends over a variable number of the adjacent subordinate veins, but at its lateral margins the swollen portion of the water tissue is sharply delimited from the cells of normal appearance which occupy most of the interval between the principal veins (fig. 5).

³ The term hypertrophy is used merely to denote an enlargement of the cells of the upper water tissue, caused by experimental procedure, which is conspicuously above the normal.

Sometimes the tension set up by these enormously swollen cells is so great that the lamina is split inward from the left margin. At such places, or in strips torn by hand from the leaf, the torn margins roll inward over the lower surface, indicating that pressure of the hypertrophied water tissue tends to depress the portion of the lamina be-

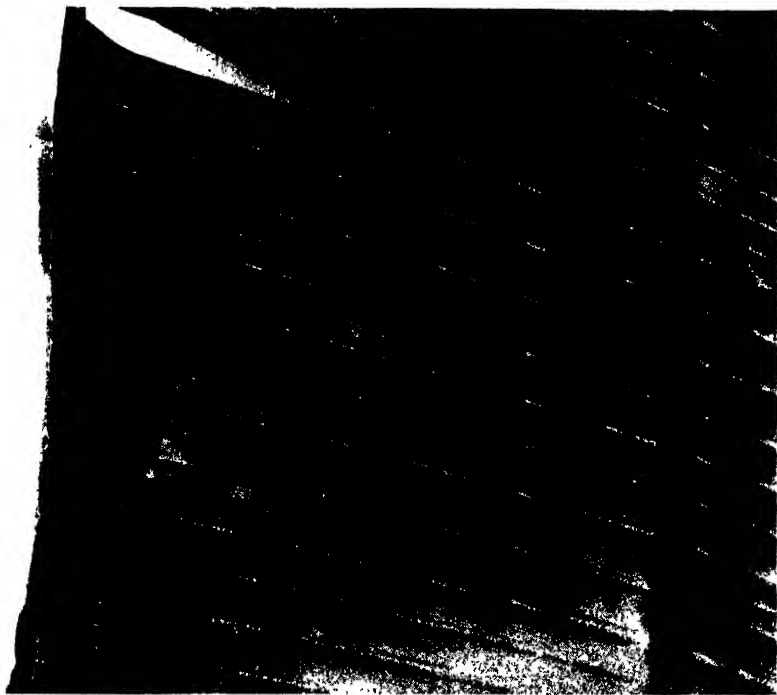


FIG. 12.—Upper surface of left side of lamina bound 12 days (note swellings above veins and backwardly rolled margin).

tween the principal veins, or to elevate the latter. The ribs formed on these bound leaves are always much stronger on the left or outer side of the lamina than on the right, and this difference persists even if the leaf is allowed to remain bound for a long period (three weeks or more). Near the right margin of a bound leaf the lamina never becomes strongly ribbed, and is in marked contrast to the opposite side. On each half of the lamina the ribbing is stronger next to the midrib than at the margin.

The thickness of the upper water tissue varies according to the

region of the lamina. Near the margins it is often single layered but usually double, near the midrib it becomes triple, and still closer to it, it is often quadruple. Immediately adjacent to a principal vein there is often one more layer of water tissue than elsewhere in the same region. The extra layer may extend as far as the fifth or sixth veinlet on either side of the principal vein. As seen in surface view, the cells of the water tissue are elongated transversely to the veins. The cell sap is perfectly clear, as in typical expansion cells, without chlorophyll or highly refractive bodies. In the bound leaves, at places where the water tissue consisted of two or three layers, the outermost layer was commonly much less hypertrophied than the one or two lying beneath it, and sometimes the cells comprising it were hardly deeper than normal. As a result of binding, the thickness of the water tissue above the principal veins may exceed that of all the remainder of the cross-section of the leaf. Thus at one point the water tissue became $480\ \mu$ deep, while the entire thickness of the lamina at this place was only $816\ \mu$. The longest cell of the water tissue at this point measured $256\ \mu$ in depth, and cells $320\ \mu$ in depth have been observed. Cells of the epidermis do not aid in unrolling, and when the leaf is bound, do not exhibit any noteworthy hypertrophy, but are passively raised by the elongating cells of the water tissue. In some places swelling of the tissue above the principal veins was so great that the epidermis was split and a fissure extended into the cushion of tissue. The lumina of the fibers lying above the principal veins are strongly extended in the anticlinal direction, so that the fibrous bundle projects far upward into the cushion of water tissue. The palisade cells immediately adjacent to the principal bundle are also elongated somewhat more than normal. The tissues on the lower side of the leaf are not affected by binding so that it cannot unroll.

On the plantation and in the jungle, one occasionally finds leaves of the Scitamineae with ribs abnormally swollen because the emerging lamina was caught in the embrace of a twining vine and unable to expand. If the plant is sickly, or if for any reason the leaf does not emerge normally and the lamina cannot unfurl at its due period, hypertrophy of the water tissue usually results. Local hypertrophy occurs wherever a sharp fold has been formed in veneration.

Prevention of the normal unrolling of the leaf also causes great hypertrophy of the water tissue on the upper side of the pulvinar bands (fig. 10). Here too the cells of the outermost layer are hardly swollen beyond the normal. The turgor of this tissue causes the lamina halves to become strongly deflexed beneath the midrib when unbound, even in moist weather and in the morning, when the other leaves are spread out horizontally; but after several days, by a process described later, they are raised to the normal position.

UNROLLING OF LAMINA IN DARKNESS

When a leaf has been constrained in the coiled condition by binding, even after a long period the right side remains yellowish, especially at the margin. The slight production of chlorophyll on this side seemed to be correlated with the very slight ribbing which was acquired, and suggested that light was essential to the unrolling of the leaf. To test this supposition, young suckers in the field were inclosed in light-proof portable houses, 90 cm. square by 2 m. high, which were equipped with light-proof ventilators at the top and bottom, and draped with white sheets to prevent their becoming too hot in the sun. The leaves which emerged in these houses unrolled normally, although they were without a trace of chlorophyll.

As in the leaves in the open, the formation of ribs began at the apex on the left side, and proceeded thence basally and to the right. The order of maturation of the expansion cells is thus an autonomic process, adjusted to bring about the harmonious unrolling of the leaf, and is not, as at first suspected, determined by the incidence of light. Their behavior in the banana is in entire agreement with the results of Löv's studies of a number of other plants. In leaves folded into a cylinder, with their edges in contact, such as those of *Luzula nemorosa*, the order in which the cells of the upper epidermis enlarge is from both margins toward the center. In simply folded leaves, such as those of *Hemerocallis*, enlargement of the expansion cells also proceeds inward from the margins. In convolute leaves the order of enlargement is from the exterior margin toward the interior. In involute leaves, as exemplified by species of the Commelinaceae, the order of enlargement is from the midrib toward both margins, while in the palms the expansion cells of the marginal folds are the first to enlarge.

Although it thus became evident that the beginning of the enlargement of the cells of the upper water tissue at the left margin was an autonomic process, not conditioned by the greater exposure of this portion of the leaf to the light, it remained to be seen whether light influences the degree of hypertrophy which can be attained by these cells. For this purpose leaves were bound as they emerged in the dark-house. After remaining bound for 16 days from the time it had completely emerged, a leaf was unbound and revealed a slight to moderate degree of hypertrophy of the cells of the upper water tissue in the neighborhood of the principal veins on the left side; but enlargement of the cells was not nearly so great as one would find in a leaf held bound for a similar period in the light. The anticlinal length of the longest cell observed was $130\ \mu$, and the 3-layered water tissue at this point was $176\ \mu$ thick, while the total thickness of the lamina was $416\ \mu$. The degree of enlargement and the turgor attained by the expansion cells are increased by light, although an enlargement normally sufficient to unroll the lamina is independent of light.

When the experiment was terminated, and the dark-houses, which were equipped with handles and could be lifted by four men, were removed from over the plants, the leaves turned green in inverse order of their age; the youngest in about 24 hours. In the leaves which had been longest in darkness, chlorophyll was produced first along the principal and stronger subordinate veins; only later in the intermediate regions. At the apex of the first leaf which had emerged in the dark-house, these veins alone turned green. These observations seem to indicate that which is confirmed by microscopical examination, that the tissues along the upper side of the principal veins are the last to lose their embryonic character, since their peculiar function demands their maturation at a later period than the rest of the leaf.

EFFECT OF PREMATURE UNROLLING

The preceding experiment suggested that the compression to which these cells are normally subjected, by their position on the concave surface of the rolled leaf, is responsible in the first place for their normal enlargement, and hence for the production of their ribs. A lamina which was just appearing above the false stem of a young sucker, and which had not yet become ribbed, was freed

by cutting away all the leaf sheaths covering it. The left side was carefully unrolled, then rerolled around the midrib and right side as tightly as possible, but with the upper instead of the lower surface facing outward. It was then bound by cord in this position and supported to relieve the strain on the still immature petiole. The left side of leaves treated in this manner never became ribbed, but the upper surface remained smooth as in immature leaves, even after being bound for 3 weeks. The right side, which was allowed to remain rolled in the normal direction, became strongly ribbed, as in the bound leaves already described.

Rerolling the leaves with the ventral surface convex places the cells of the upper water tissue in an unnatural position, and it was desirable to determine the effect of merely flattening out the lamina. This experiment was attended by considerable practical difficulty. The framework which supports the lamina must hold it perfectly flat, and yet permit it to slip upward as the sheath of the leaf elongates. The leaf used must be of exactly the right age, for if too young it will be unable to withstand the exposure to which it is subjected by premature unrolling, and if too old, will already have begun to become ribbed. Finally these requirements were met by constructing a framework consisting of two parallel series of bars of split bamboo. A lamina about 1 m. long, which had emerged for one-quarter to one-half its length, from a young sucker, was chosen. It was freed of the surrounding leaf sheaths, the left side unrolled, the framework slipped over it and set into the ground beside the plant. During the first few days, or until the tissues had become green, it was shaded by tying cut banana leaves to the outside of the frame. The left side of these leaves became at most very slightly ribbed, always much less than in normal leaves. In small restricted areas, which had not been held perfectly flat in the frame, the ribs were much stronger, and in some places the expansion cells were even somewhat hypertrophied, showing that a slight concavity of the upper surface is sufficient to cause ribbing.

Leaves which were prematurely unrolled and did not become normally ribbed, usually hung more or less limp from their midribs instead of being spread out horizontally in the morning and evening, and on wet days. The ribs are necessary for the mechanical support

of the broad flat lamina, and the expansion cells, in addition to the function of unrolling the lamina, must create the framework for its support.

EFFECT OF REROLLING MATURE LEAVES

Two mature leaves, the youngest completely unfolded leaf, and the one next below this on a young sucker, were loosely rolled into the position of vernation and held by binding with cord. After 9 days they were unrolled and examined. The younger leaf had been affected by this treatment much more than the older. The water tissue above and beside the principal veins of the former was strongly hypertrophied, and the leaf much more conspicuously ribbed than normal. When the water tissue was three layers thick only the inner two layers were hypertrophied; when two layers thick, only the inner. The hyaline cells on the upper side of the pulvinar bands, particularly on the right side, which was the more strongly inrolled, were also considerably hypertrophied. The older leaf showed the same symptoms in a reduced degree.

DEVELOPMENT OF PULVINAR BAND

At night, in the early morning and late afternoon, and continuously on wet days the two sides of the lamina stand out in a plane on either side of the midrib. In the middle of bright sunny days they bend beneath the midrib, and eventually, if the day is dry the dorsal surfaces of the two sides are brought into contact. Since the stomata are situated principally on the lower side of the lamina, and are thereby brought into a more protected position, and moreover because by the assumption of the profile position the absorption of sunlight is greatly decreased, the water loss must be considerably diminished, although at present no actual measurements on this point are available. When a leaf has been torn into strips by the wind, the individual strips behave as though the lamina were still intact. If the sky suddenly becomes overclouded or there is an unexpected shower during the middle of a day which was at first sunny, recovery of the horizontal position by the lamina halves is rapid.

That the halves of the lamina do not passively sink down as the result of wilting may easily be demonstrated by holding such a leaf in an inverted position, when the lamina halves will not droop down

but remain folded upward. Their movements are determined by changes in the turgor of the pulvinar bands which border the midrib on each side, and the flexure is sharply localized in these bands. It therefore becomes of interest to examine their structure. Their width is about 5 mm. in large leaves, and in thickness they taper from about 1.8 mm. next the midrib to 1.2 next the lamina half. Fig. 7 represents a portion of the cross-section of a pulvinar band of a leaf of medium size, which was just appearing from the false stem. It shows the condition found in the mature leaf tolerably well, except in the form of the cells lying immediately below the upper and above the lower epidermis. The change which occurs in the cells of the upper water tissue during the process of expansion has already been described. The central portion of the organ is characterized by the absence of lacunae (although small spaces between the rounded cells are numerous), the many vascular bundles, and the conspicuous sheaths of fibers which accompany them. Toward the lower surface bast bundles are numerous, surrounded by exceedingly thick sheaths of fibers, and still lower in the cross-section strands of fibers alone are found. Above the lower epidermis two layers of collenchymatously thickened cells occur. It is the behavior of these cells, and those immediately above them, which is of particular interest.

When the leaf first expands, the great turgor engendered in the upper water tissue of the pulvinar bands causes the halves of the lamina to become reflexed below the midrib (fig. 1). After a few days the lamina becomes flattened out again. This is brought about by the gradual enlargement of those cells on the lower side of the pulvinar band which lie between the hypodermal layer and the lowest of the bast bundles. Their elongation does not begin until the leaf has expanded, and is completed in about 10–12 days. The process of elongation was studied by removing with a sharp scalpel little rectangular pieces from the apex, middle, and base of each pulvinar band of a single leaf at intervals of 4 days. The leaf was so large that the loss of these small pieces of tissue did not seem to affect it, and successive samples in each region were spaced a few centimeters apart, so as to avoid wound reactions. The thickness of the tissue formed by these elongating cells, and the length (anticlinal) of the longest cell in each region, were measured by a micrometer scale,

and the results of one series of measurements are recorded in table I. In the mature leaf the tissue is composed of enormously elongated, irregularly prismatic cells, with clear contents and containing few chloroplasts (fig. 8).

Very young leaves do not respond to changes of atmospheric conditions by rising and sinking. The diurnal movements do not become

TABLE I
DEVELOPMENT OF PRISMATIC CELLS OF PULVINAR BAND; TOTAL
THICKNESS OF PRISMATIC TISSUE IN MICRONS

POSITION	March 1*	March 5	March 9	March 13	March 25
Left side					
Apex.	64	224	272	336	384
Middle . . .	96	304	400	544	512
Base.	96	320	416	528	496
Right side					
Apex.	80	272	304	400	448
Middle . . .	96	368	400	512	512
Base	96	384	400	400	496
Length of longest cell in three sections					
Left side					
Apex.	26	122	130	204	204
Middle . . .	26	115	207	229	233
Base.	30	144	267	241	215
Right side					
Apex.	30	155	163	222	233
Middle . . .	26	144	192	241	218
Base	26	144	174	167	207

* On March 1 the base of the lamina had just become free from the false stem; the cells on the lower side of the pulvinar band were not in the least elongated, but appeared as in fig. 7.

pronounced until after development of the prismatic cells. The movements seem to be the result of variations in turgor of the two antagonistic layers, the upper water tissue and the lower prismatic tissue. In a segment of a cut leaf the latter loses water more rapidly, and the lamina halves bend downward beside the midrib. Placed in water, the prismatic cells rapidly regain their turgor, and the lamina halves are again spread in a plane.

The conditions necessary for development of the prismatic cells are pressure and light. The pressure is normally supplied by the antagonistic action of the upper water tissue, which, by its strong

development when the leaf first expands, bends the pulvinar band downward and throws the cells on its lower side into a state of compression. In the experiments in which unrolling of the leaf was prevented by binding, the cells on the lower side of the pulvinar band did not become prismatic. When the left side of the lamina was unrolled and then rerolled in the reverse direction, development of prismatic cells on this side was very irregular, because in bending the leaf back the flexure generally occurred in the lamina itself rather than in the thicker pulvinar band, and the lower surface of the latter only occasionally became concave. On the right side in these experiments no prismatic cells were formed. When the time of unrolling of the lamina was delayed a week or two by binding, and the water tissue on the upper side of the pulvinar band was greatly hypertrophied, the lamina halves became strongly reflexed after the cord was removed, but gradually bent upward as the prismatic cells developed. Leaves which expanded in darkness never developed prismatic cells so long as they were left in the dark-house. Since, as a result of the slighter enlargement and turgidity of the cells of the upper water tissue of the pulvinar band, the leaves which unfold in darkness never become so strongly reflexed along the midrib as leaves which emerge in the light, there was a possibility that failure of development of the prismatic cells was a result of insufficient compression of the lower side of the pulvinar band. Leaves were bound as they emerged in the darkness and the unfurling delayed a week or more, which resulted in a slight hypertrophy of the upper water tissue, and when unbound the lamina halves became more strongly reflexed. Even under these circumstances there was no development of prismatic cells in darkness. The appropriate cells retain their capacity to elongate, however, and even after their development has been delayed 20 days by darkness, when returned to the light they enlarge and become prismatic.

In all of the species which I have been able to examine, including *Musa textilis*, *M. tomentosa*, *M. malaccensis*, *M. sanguinea*, *M. rosea*, and many varieties of *M. sapientum*, the pulvinar band with its prismatic cells is well developed, and the lamina halves fold downward as the leaf loses water. Although there is great development of fibers, undoubtedly made necessary by the strategical position of the

organ, in the pulvinar bands of all of these species they are in general weakly or not at all lignified. The same strand of fibers is usually strongly lignified on both sides of the pulvinar band, that is, in the midrib and the lamina half, but in the band itself it is either not lignified or its lignin reaction is much weaker than in the adjacent regions. The failure of lignification is typical of pulvini, and is of advantage to an organ which must bend repeatedly. I have already described (5) a peculiarity of the cuticle over the pulvinar band of *M. sapientum* which seems associated with the motility of this organ.

The genus *Musa* is the only member of the Scitamineae known to the writer the leaves of which fold downward in dry weather. In all others (including the species described later) the leaves fold upward, exposing the lower surface, in which the stomata are principally situated. In these species there are no distinct pulvinar bands, and the bundles of fibers are continuously lignified as they pass from the midrib to the lamina halves. Like the majority of monocotyledonous leaves, these reverse the movements of unfolding as they dry (3). GOEBEL (2) points out that the movements of plant organs must be considered primarily in relation to their development, external and internal symmetry, and mode of unfolding. An organ performs movements because its structure is asymmetrical (whether or not the lack of symmetry is externally evident), and opposite sides are differently affected by various external or internal changes. The movement may be performed first as the organ expands, and subsequent movements of the mature organ may be merely reversals and repetitions of the act of unfolding. Since the movements of plants are determined by structural peculiarities which frequently originate in response to needs or conditions unrelated to these movements, they may or may not happen to prove useful, but are not of necessity useful. This certainly is true of the movements of many monocotyledonous leaves in dry weather. The leaf tends to return to the position it occupied in vernalion, because the expansion cells, which by their turgor spread out the lamina halves and hold them so, lose their turgor and permit the antagonistic forces of the tissues on the opposite side of the leaf to reverse the process of unfolding. Far from being of advantage to the plant, these movements appear just the reverse, for the stomata are thereby more exposed. But because of

development of the prismatic tissue of the pulvinar bands, movements of the lamina in dry weather are not mere reversals of the act of expansion, and appear to be advantageous to the plant. Like the sleep movements of the leaves of *Tropaeolum majus*, which bend outward at night although the upper surface is directed inward in the immature leaf, they are exactly opposite to the movements of unfolding. The prismatic cells of the pulvinar band appear to be a late acquisition in the phylogeny of *Musa*, since its nearest relatives, *Ravenala* and *Heliconia*, lack them; they are certainly a late development in the ontogeny, and originate only under special conditions of pressure and light.

Unfolding of leaves in other genera of Scitamineae

MUSACEAE

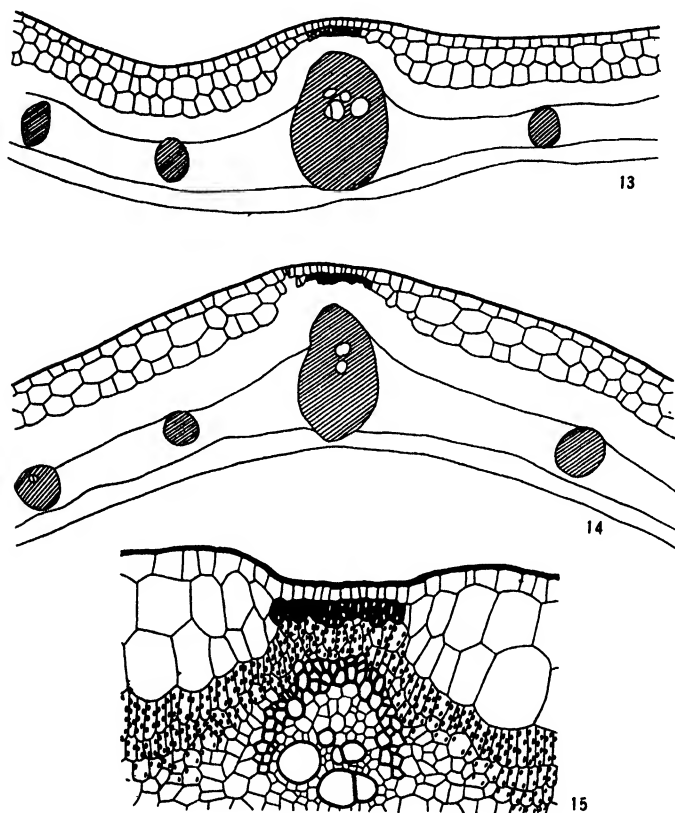
Heliconia bihai L.—The long, slender leaves of this plant emerge very gradually from the short false stem, and the apex has usually spread out long before the base has escaped from between the sheaths of the older leaves. The upper water tissue is everywhere (except close to the midrib) but a single layer in thickness. Unrolling of the lamina halves and formation of transverse ribs proceed almost exactly as in *Musa*. The prevention of unrolling by binding with cord caused the water tissue above and immediately adjacent to the principal veins to become enormously swollen, forming thick welts running across the lamina half. Single cells reached $432\ \mu$ in anticlinal length, about 3-4 times the normal depth. The thickness of the hypertrophied water tissue often exceeded that of the remainder of the leaf, and at one place, where the total cross-section was $560\ \mu$, it occupied $352\ \mu$. The water tissue above many of the stronger subordinate veins was hypertrophied, and in places where the upper surface was particularly concave (the lamina does not form a perfect helix in vernation) the hypertrophy extended completely across the interval between the principal veins.

In dry weather the leaf folds upward, exposing the stomata, as do those of all other species of *Heliconia* with which I am familiar.

ZINGIBERACEAE

Alpinia exaltata (L.) R. & S.—The aerial shoots of this species, attaining 5 m. in height, consist of a slender stem everywhere sur-

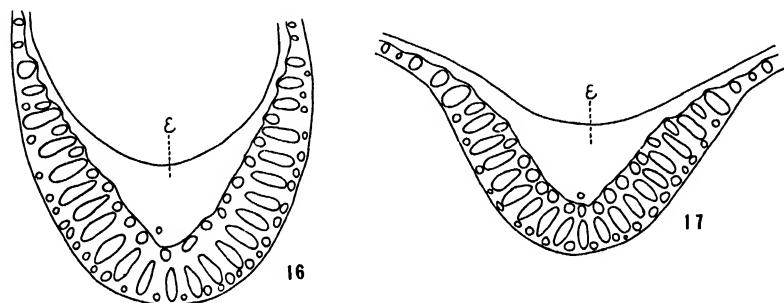
rounded by many thicknesses of the long overlapping sheaths, and are continued upward into a false stem composed of these sheaths alone. When a new lamina emerges it is practically full-grown. If such a leaf is unrolled, it is found to be perfectly flat and without



FIGS. 13-15.—*Alpinia exaltata*: Fig. 13, cross-section through principal vein of leaf just beginning to emerge; only water tissue and epidermis shown in detail; camera lucida $\times 50$; fig. 14, same, from fully expanded leaf; $\times 50$; fig. 15, cross-section through upper portion of principal vein of leaf bound 14 days; $\times 112$.

ribs, but it feels slightly rough if the finger is drawn across it transversely to the veins, which project slightly above the surface (fig. 13). Even the strongest veins do not occupy the entire interval between the upper and lower water tissues, as in *Musa*, but the palisade tissue is continuous across them. The two-layered upper water tissue

is interrupted above the stronger veins by a group of small cells, the walls of which become thickened even before the leaf appears at the top of the false stem. For this reason the process of unrolling differs from that in *Musa* and *Heliconia*. The cells of the water tissue on either side of the principal veins are the chief expansion cells, and by their enlargement cause the lamina to become strongly ribbed (fig. 14). Prevention of unrolling by binding does not cause the enormous hypertrophy characteristic of all the other Scitamineae studied, probably because the water cells are interrupted by cells which are thick walled and incapable of elongating, and hence hold the others



FIGS. 16, 17.—*Alpinia exaltata*: fig. 16, cross-section through midrib of leaf before expansion; fig. 17, same, from expanded leaf; *e*, expansion cells; $\times 5$.

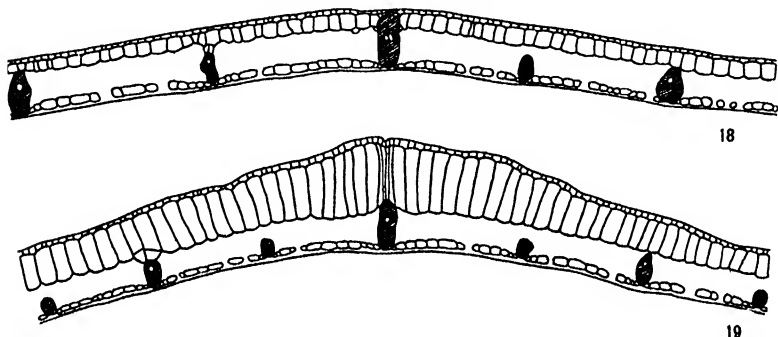
back. There is, however, a definite though small hypertrophy of the upper water tissue extending completely across the interval between the principal veins. Fig. 15 shows the region above a principal vein of a leaf which had been bound for 2 weeks. The water tissue bulges up above the mechanical tissue on either side, but appears to be held in check by the attachment of the epidermis to the latter. The outer side of the bound lamina exhibits other symptoms of the high turgidity of its expansion cells, such as splitting of the tissue and rolling backward of the margin, and feels smoother and more turgid than the normal leaf.

Fig. 16 shows how the clear transparent expansion cells occupy the entire central portion of the cross-section of the midrib, while the vascular bundles are all crowded to the lower side. At the proper time the former increase in size and turgidity, spread the midrib, and flatten out the lamina halves (fig. 17). On drying, the lamina halves

fold together again, as a result of the loss of turgidity by these strategically situated cells. The stomata, situated chiefly in the lower surface of the leaf, are thereby exposed.

CANNACEAE

Canna sp.—The *Canna* chosen for study is a species with small red flowers commonly cultivated about the province of Bocas del Toro, Panama, and often found growing spontaneously. It is apparently undescribed, and in the unsatisfactory state of the taxonomy of the genus it appears undesirable to make a new species of it without a revision of the group. The leaves of *Canna* species show great uni-



FIGS. 18, 19.—*Canna* sp.: fig. 18, cross-section through principal vein of normally expanded leaf, showing water tissue; fig. 19, same, from leaf bound 7 days; $\times 25$.

formity in structure, however, so specific identity is not important for the present purpose.

Canna is the only genus of the Scitamineae studied by Löv, or about which there is any published account of the mode of unrolling of the lamina. Löv recognized that the water tissue furnishes the principal expansion cells, but failed to notice the greater enlargement of those above and adjacent to the principal veins, which results in the formation of the ribs so essential to the mechanical support of the lamina throughout the order. The epidermis plays an inconsiderable part in the act of unrolling. Löv also gives some interesting observations on the substitution of other tissues in the act of unrolling when the water tissue is destroyed. By scratching the upper surface, with a sharp knife, it is possible to injure the water tissue without destroying the epidermis. In this case the latter cells become

much deeper than normal, and the leaf continues to unfurl. When the epidermis and water tissue are both destroyed by touching with a glowing needle, an abnormal growth of the palisade cells takes place, particularly in the neighborhood of the large veins, where periclinal divisions of the cells occur. The leaf manages to unroll.

The leaf which is just beginning to emerge is unribbed. Formation of the weak ribs is accomplished without the cells of the single layered water tissue becoming much larger above and adjacent to the principal veins than midway between them (fig. 18). Prevention of unrolling by tying caused the great hypertrophy of the cells of the upper water tissue (fig. 19). It was most pronounced above the principal veins, but extended completely across the leaf, with lesser maxima above many of the stronger subordinate veins. The lamina became strongly ribbed. The hypertrophied cells of the water tissue measured as much as $384\ \mu$ in depth, which is 4-5 times the normal, and accounted for over half the cross-section of the leaf. The epidermal cells were also greatly enlarged, becoming in places $67\ \mu$ in depth, as opposed to $15-26\ \mu$ in the normally unfurled leaf. The leaves curled backward when unbound and placed in water.

The leaves of *Canna*, like those of *Musa*, unroll normally in darkness.

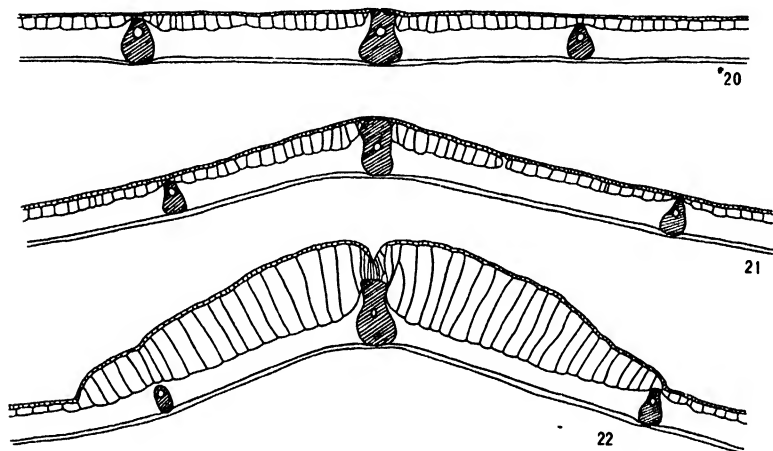
MARANTACEAE

Calathea magnifica Morton & Skutch.⁴—The leaves of this newly described species are ovate-oblong and very large, reaching 114 cm. long by 58 cm. broad. At the time the lamina appears above the short false stem, and even after the apex has begun to turn green, the upper surface is perfectly smooth, without the ribs so conspicuous in the mature leaf (fig. 20). The fibers on the upper side of the vascular bundles are thin walled, while those on the lower side are already conspicuously thickened. The water tissue is single layered everywhere except in the immediate vicinity of the midrib. It is usually not continuous above the principal veins; the fibers on their upper side are either in direct contact with the epidermis or separated from it by a few small scattered cells which appear to belong to the water tissue. In unfurling, the cells on either side of the principal

⁴ Jour. Wash. Acad. Sci. 20: 372. 1930.

veins enlarge considerably, with the result that the leaf becomes ribbed (fig. 21).

Prevention of unrolling by binding the leaf causes great hypertrophy of the cells of the water tissue in the neighborhood of the principal veins. The region of hypertrophy is laterally sharply delimited from the surrounding water tissue (fig. 22). Single cells $464\ \mu$ in depth were observed, 2.5–3.7 times the depth found in normal leaves. Where it was thickest, the water tissue accounted for two-



FIGS. 20–22.—*Calathea magnifica*: fig. 20, cross-section through principal vein of leaf before expansion, showing water tissue; fig. 21, same, through normally expanded leaf; fig. 22, same, through leaf bound 8 days; $\times 23$.

thirds to three-fourths the entire cross-section of the lamina. There was generally a deep furrow in the cushion of tissue above the center of the vein, except in the rare cases where the water tissue was continuous above it. This occurred because the epidermal cells, here in contact with the fibers, were unable to elongate as rapidly as the cells of the water tissue, which for this reason bulged above them. The epidermal cells in this position did undergo an enormous elongation, becoming $44\text{--}78\ \mu$ in depth when normally they were $7\text{--}11\ \mu$, but they appeared to have been passively stretched by the water tissue (fig. 22).

As in the other species described, not only must the two halves of the lamina unroll individually, but it is necessary that they bend

outward along the sides of the midrib. This is accomplished by the great development of prismatic expansion cells, with clear cell contents, in these regions. The largest vascular bundles are situated near the upper side of the organ, and numerous smaller ones are scattered beneath (fig. 23). The expansion cells penetrate deeply between the largest bundles, and by their enlargement at the time of unfurling of the leaf bend back the lamina halves. In drying they lose their turgor, and the lamina halves bend upward. In the middle of sunny days it is striking to see the large leaves of these plants, which grow in dense stands in low open places, all folded together upward, exposing the white, wax encrusted lower surface, in which most of the stomata are situated.

Summary

1. An experimental study was made of the mechanics of the unrolling of the leaves of *Musa* and *Heliconia* (Musaceae), *Alpinia* (Zingiberaceae), *Canna* (Cannaceae), and *Calathea* (Marantaceae), representing each of the four families of the tropical order Scitamineae.

2. The large laminae of all the species studied are convolute in veneration. They are practically full-grown at the time they begin to emerge from the top of the false stem, which, to a greater or less degree, is characteristic of most of the genera of this order. At this time, if the lamina is unrolled, it is found to be smooth and flat, without the transverse ribs so characteristic of these plants.

3. Unrolling is effected by the timely enlargement of the cells of the upper water tissue above and immediately adjacent to the principal veins. The localized enlargement of these cells results in raising the principal veins above the general surface of the lamina, thus forming the ribs. In the coiled position of the leaf they are pushed inward, come to occupy a curvature of smaller radius, and are thus

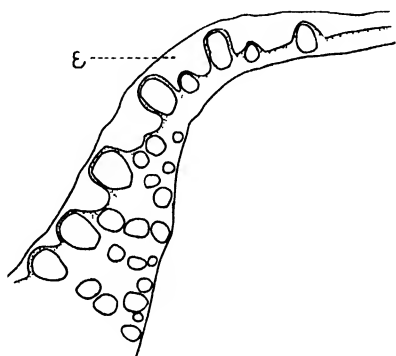


FIG. 23.—*Calathea magnifica*: cross-section through junction of midrib and lamina half; e, expansion cells; $\times 12$.

(with the turgid cells which lie above them) brought into a state of compression. In this compressed state they force the lamina to unroll.

4. In *Musa* the fibers on the dorsal side of the veins are thick walled and become lignified somewhat before the portion of the leaf in which they lie begins to unroll. The fibers on the ventral side remain thin walled until the leaf has unfolded, and never become lignified.

5. Enlargement of the expansion cells proceeds inward from the outer margin of the lamina, and from the apex toward the base.

6. If the unrolling of the leaf is prevented by binding it with cord, the expansion cells in all species studied except *Alpinia* become greatly enlarged, and occupy over half the entire cross-section of the lamina. In *Alpinia* the hypertrophy is relatively slight, apparently because the water tissue is interrupted by thick walled cells which are not able to stretch. Only in *Canna* does the hypertrophy extend completely across the lamina half; in most cases it is strictly limited to the region of the principal and the stronger subordinate veins.

7. Leaves of *Musa* and *Canna* were found to unroll in complete absence of light. In darkness, however, the hypertrophy of the water cells of bound leaves is much less than of those in the light.

8. If the lamina of *Musa* is prematurely unrolled and held in a plane, the expansion cells fail to enlarge. They enlarge only when in a state of compression.

9. The ribs are essential to impart rigidity to the lamina halves, which are otherwise without lateral support.

10. In addition to the unrolling of each side of the lamina, a special arrangement is necessary to bend it outward from the midrib. This is effected by great development of expansion cells along the edges of the midrib (*Musa*, *Heliconia*, *Calathea*), or in the center of the midrib (*Alpinia*).

11. In most members of the Scitamineae, when the leaf suffers great water loss and the expansion cells lose their turgidity, the process of unfolding is in part reversed. Each side of the lamina bends upward along the midrib, exposing the stomata, which are situated principally in the lower surface. This non-adaptive movement is a necessary result of the structure and method of unfolding of the leaf.

12. In *Musa* the sides of the lamina fold downward in dry weather. This is a result of the formation of a rather massive tissue of prismatic cells in a strip along either side of the midrib, which thus becomes a pulvinar band. The rising and falling of the lamina halves are brought about by differential changes in the turgor of two antagonistic tissues, the expansion cells on the upper side and the prismatic cells on the lower.

13. The prismatic cells begin to develop only after complete expansion of the leaf, when stimulated by the compression to which they are subjected after the expansion cells on the upper side of the pulvinar band bend it downward. Hence they do not develop in leaves which are prevented from unfolding by binding. They develop only in the light.

In conclusion, I wish to express gratitude to Professor DUNCAN S. JOHNSON for his continued interest in the course of my tropical studies; to Dr. JOHN R. JOHNSTON, Director of Agricultural Research of the United Fruit Company of Boston, for allowing me the use of the Company's research stations in Central America, and for many other courtesies extended to me by the Company; and to Mr. JOSEPH H. PERMAR, Director of the station at Almirante, Panama, where the present study was completed, for his unfailing cooperation in my work.

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POLLEN TUBE GROWTH AND CONTROL OF GAMETOPHYTIC SELECTION IN COCKLEBUR, A 25-CHROMOSOME DATURA¹

JOHN T. BUCHHOLZ AND ALBERT F. BLAKESLEE

(WITH THIRTEEN FIGURES)

Introduction

Among the primary ($2n+1$) types of *Datura stramonium*, several have been found which may be transmitted through the pollen in back-crosses to normal $2n$ plants. We have selected Cocklebur (Ck) as one for special study, together with Wedge (Wd), its secondary.

POLLEN TUBE GROWTH FOR COCKLEBUR POLLEN

An analysis by means of pollen tube growth tests of the pollen from Ck reveals distinctly the presence of two classes of pollen tubes. Figs. 1-4 are made from counts of the ends of pollen tubes in the style after 12 hours' growth; figs. 7 and 8 are similar tests after 16 hours' growth. The normal appearing pollen tubes are plotted above the datum line in the appropriate 2 mm. interval² corresponding to their distance from the stigma, while the abnormal appearing pollen tubes are plotted below the datum line. The shaded vertical bar at the left in each diagram indicates the number of pollen grains remaining ungerminated on the stigma. Aborted pollen grains could be observed but could not be counted reliably on the microscopic preparations and are not included, but their proportion has been determined by CARTLEDGE (4).

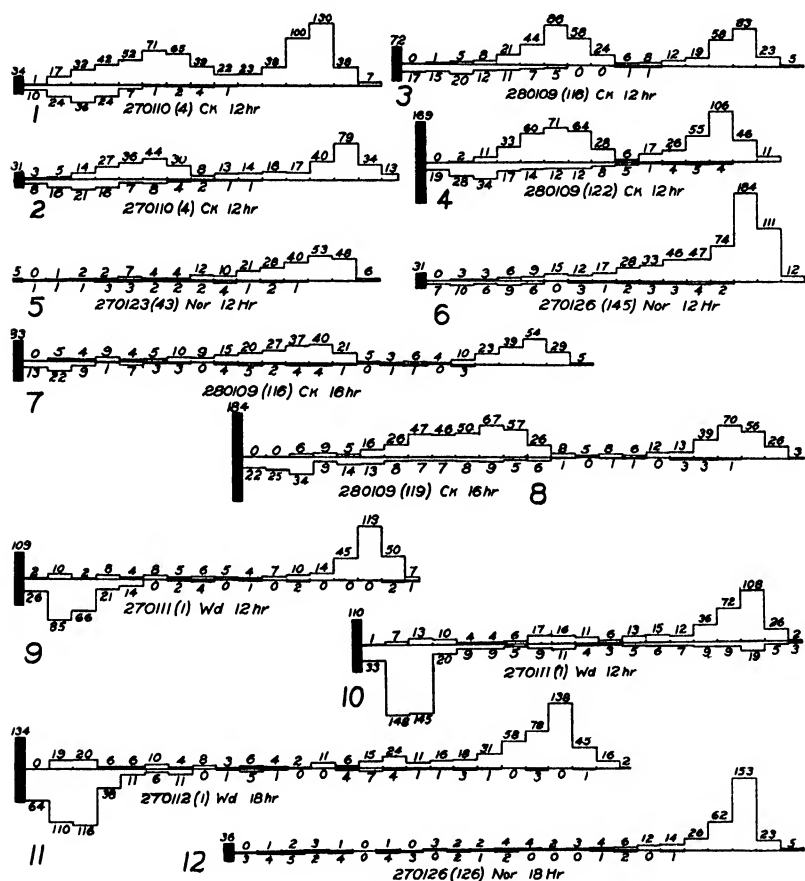
The figures of the Ck tests all show a distinct bimodal grouping; two groups of tubes of nearly equal proportions, when we allow for the usual proportion of overlapping of stragglers from the longest group which may be included in the second group. The inference from an examination of these distributions is that these are the two

¹ This cooperative investigation was made possible by grants from the Joseph Henry Fund of the National Academy of Sciences.

² In some previous publications we have used 3 mm. intervals for plotting, and in others (10), including this paper, we are using 2 mm. intervals.

classes of pollen tubes, with $(n+1)$ and n chromosomes respectively.

Figs. 5 and 6 represent similar tests made as controls, in which the pollen of $2n$ plants was used. Here we have pollen tubes with n



FIGS. 1-12

chromosomes which are grouped chiefly in a single mode of distribution at a distance which corresponds to the position of the longest groups in figs. 1-4. The inference is, therefore, that the n pollen tubes in figs. 1-4 make up the first or longest group, while the $(n+1)$ pollen tubes make up the second group. This distribution indicates that either the $(n+1)$ pollen tubes germinate after several

hours' delay, or they grow more slowly. We have determined these points somewhat roughly and found, by comparing and plotting these two modes in a series of tests after various successive time intervals, that both the period of germination is prolonged and the rate of growth of these ($n+1$) pollen tubes is slower than for those with n chromosomes. At a temperature at which the growth rate of the first mode was 2.6 mm. per hour, that of the second mode was 1.9 mm. per hour. The delay in germination and slower growth seem to place the group in the second mode at less than half the distance reached by the first, in figs. 1-4.

In figs. 1 and 2, from tests made in 1928, only 5-6 per cent of the pollen grains remained ungerminated on the stigma, but in figs. 3 and 4 of the following summer 11.5 and 19.5 per cent respectively remained ungerminated. The relative proportion of pollen tubes in the two groups is not greatly modified, however, nor is the proportion of burst pollen tubes altered consistently in these or the 16-hour tests of figs. 7 and 8. Figs. 1 and 2 show tests made under very favorable conditions of pollen development, when the styles used for testing as well as the pollen parents seemed to be at their best, while figs. 3, 4, 7, and 8 were made when these conditions were somewhat less favorable. It is obvious that under the conditions of either of these tests the ($n+1$) pollen tubes may reach the ovary in considerable proportions, since the style usually remains attached for two or more days after pollination.

If a great amount of pollen is applied to the stigma in making pollinations, the slow growing ($n+1$) tubes will have only a small chance of accomplishing fertilization, because the pollen tubes with n chromosomes may fertilize all of the eggs before the arrival of the ($n+1$) group. In making routine pollinations the pollen is usually crowded. If only a medium or a small amount of pollen (400-500 grains) is used in making the pollinations, however, there is some chance for fertilization by ($n+1$) pollen tubes, since the ovary of a well developed flower may have 400-600 ovules. The question has also been tested with the ($2n+1$) Globe, in male back-crosses using counted pollinations, when the optimum number of pollen grains for the transmission of the extra chromosome was found to be around 500 (14, 15).

We have frequently found evidence of pollen transmissibility of Ck in connection with routine breeding experiments. Some of these are given in table I. Not only is Ck transmitted through the pollen from Ck plants, but its secondary Wd transmits the Ck through the pollen, while the Wd itself is generally not transmitted. This relationship between Ck and Wd will be considered further in a later paragraph.

SPECIAL POLLINATION METHODS AND EXCISION OF STYLES

Late in the summer of 1927 it was decided to put to a more critical test the question of pollen transmissibility in Ck, in the hope of de-

TABLE I

POLLEN TRANSMISSIBILITY OF COCKLEBUR AND WEDGE IN GARDEN PLANTS
FROM ORDINARY CROSSES WITH FOUR LINES

	COCKLEBUR						WEDGL						
	Ped. no.	Plants record- ed	Nor- mal	Ck	Per cent	Wd	Ped. no.	Plants record- ed	Nor- mal	Ck	Per cent	Wd	Per cent
Line 21...	230231	181	171	10	5 5	0	230232	180	170	8	4 4	2	1.1
Line 26 .	230250	174	166	8	4 6	0	230251	180	180	0	0 0	0	0
Line 33 .	230268	151	128	23	15 2	0	230269	199	191	8	4 2	0	0
Line 36 ...	230291	102	86	16	15 7	0	230292	105	103	2	1 9	0	0
Totals.		608	551	57	9 2	0	Totals ...	664	644	18	2.7	2	0.3

veloping experimental methods by which we might increase or decrease this transmissibility. Pollen tube growth tests made late in August showed good pollen germination and a striking bimodal grouping of the pollen tubes, indicating favorable conditions of pollen at the time of making these tests. In order to control pollen tube growth conditions as far as possible, potted plants from line 1A were used and kept at a uniform, moderately low temperature in the basement of the laboratory during the period of pollen tube growth. Favorable pollen parents were used and the condition of temperature was similar to that during the earlier tests, including those represented in figs. 1 and 2.

The methods chosen for control of the competition between pollen tubes were those used in our studies of pollen transmissibility of ($2n+1$) Globes. One of these consists of restricting the pollinations to a small or medium number of pollen grains (15). With this meth-

od we combined style-excision operations, the styles of some of the flowers being cut off after the longest pollen tubes had entered the ovary, in order to exclude the second slow growing group. The pollen grains were not counted, but the numbers were roughly estimated as the pollinations were made, by using small camel's-hair brushes and examining these under a Greenough binocular microscope in order to estimate the quantity.

While the 2*n* plants used as females were potted plants, the pollen used was taken from Ck plants growing in the garden whose pollen had been showing at the time a high percentage of germination and favorable conditions of pollen tube growth.

Three parallel sets of pollinations were made, beginning on an afternoon, and all plants pollinated were kept under similar conditions. Flowers of set A were used for examination of pollen tube growth in the style from time to time on the morning of the following day, in order to determine the progress of the longest group of pollen tubes and to predict the time of their arrival at the ovary in the remaining plants. Those of set B were left undisturbed, and those of set C were used for style-excision experiments. The styles of set C were cut off when the longest group of pollen tubes had entered the ovary, as predicted by examination of rapidly prepared tests of set A. This operation excluded the second or slow growing group illustrated in figs. 1 and 2. Actually it was found that we had excised most of the styles a little too soon; and while the parallel set A indicated that the ends of the pollen tubes had actually entered the ovary (which was also borne out by a later experiment of the styles cut from set C), it appeared that many of the pollen tubes were so shocked or injured in this operation that they failed to continue growth; thus the seed yield was lower than desired.³

While the seed yields from the style-excision experiments were low, it is obvious that we were successful in admitting only a part of

³ Perhaps we should have allowed more for the distance back from the tips of the pollen tubes to the point where the callose plugs have formed in order to prevent backward "bleeding" of pollen tube protoplasm. The callose plugs in normal plants begin to form about 6 mm. back from the ends of the tubes, and occur at average intervals of 1.6 mm. A pollen tube 30 mm. long would have a newly formed callose plug about 6 mm. from its forward end and others at intervals of about 1.6 mm., with the oldest callose plug about 3.5 mm. below the top of the stigma.

the first group and in excluding all of the second group, for table II, set C, shows that all the plants obtained from these seeds were normal. This shows that the first group are n and not $(n+1)$ pollen tubes.

Table II also shows the success obtained in set B in lessening the competition among pollen tubes for fertilization of eggs. The restricted pollinations prevented the first group of pollen tubes from monopolizing the ovules, so that there were some remaining to be fertilized by the second group. The proportion of Ck plants obtained from most of these seed capsules was as high as or higher than that usually obtained in self-pollinated Ck capsules, ranging as high as 34.1 per cent in no. 24a, and giving an average of 27.6 per cent. They averaged about three times the proportions obtained in backcrosses made by the usual routine methods indicated in table I. The difference between these (table II B) and the style-excision experiments (table II C) shows that the slower group of pollen tubes are the ones which carry the extra chromosome necessary to produce the Ck ($2n+1$) type.

DIFFERENCES IN PROPORTIONS OF Ck PLANTS IN UPPER AND LOWER PARTS OF SEED CAPSULES

There is still another set of data obtained which is in agreement in identifying the slower group of pollen tubes as made up of $(n+1)$ tubes, namely, the comparison of the upper and lower portions of the seed capsules. The pollen tubes which are first to arrive in the interior of the ovary fertilize the ovules near the top, while those which arrive later accomplish fertilization in ovules farther down. We have occasionally observed that if only a limited quantity of pollen is applied to the stigma, the lowest ovules are the ones which fail to enlarge, presumably through lack of fertilization.

Several of the capsules in these tests were not divided. No. 14a was too small to warrant a division into upper and lower half, and 13a was harvested in a state too mature to be divided. The other three capsules of set B were carefully divided into upper half and lower half and grown as separate pedigrees (table II). In every case where the capsule was divided there was an overwhelming increase in the proportion of Ck types in the lower portion of the seed capsules compared with the upper portion. Capsule 24 had 65.4 per

TABLE II
RESULTS OF RESTRICTED POLLINATIONS IN COCKLEBUR MALE BACK-CROSSES USING EXCISION OF STYLES AND
DIVISION OF SEED CAPSULES INTO UPPER AND LOWER PORTIONS

PARENTS	SET B SPARINGLY POLLINATED, STYLES NOT EXCISED						SET C SPARINGLY POLLINATED, STYLES EXCISED			
	Capsule no	Planting no	Seeds planted	Plants recorded			Percentage Cocklebur			Seeds planted
				Total	Normal	Ck	U $\frac{1}{2}$	L $\frac{1}{2}$	Whole capsule	
26or(1)X 26or(8)(ir8)	U $\frac{1}{2}$ 24a { L $\frac{1}{2}$	271011 271012	200 235	188 205	188 71	0 134	0 0	65 4	34 1	27972
26or(4)X 26or(8)(ir6)	13a 14a	271009 271010	146 86	132 85	120 58	12 27	0 0	0 1 31 8	9 1 31 8	271008
26or(4)X 26or(8)(ir8)	U $\frac{1}{2}$ 25a { L $\frac{1}{2}$	270975 270976	162 178	147 158	141 75	6 83	4 1 0 6	52 5	29 2	
26or(5)X 26or(8)(ir8)	U $\frac{1}{2}$ 26a { L $\frac{1}{2}$	271013 271014	175 173	167 167	166 85	1 82	0 6 1 4	49 1	24 9	27973 27974
Totals...	1355	1249	904	345	1 4	56 4	27 6	133

cent Ck in the lower half and none in the upper, while capsules 25 and 26 had about half Ck types in the lower half. Thus by this method have been obtained pedigrees with twice the proportion of Ck types found in ordinary selfed capsules, or in pedigrees obtained by any other known method. This is remarkable in view of the rule that most $(2n+1)$ types are usually not transmitted through the pollen, and emphasizes the importance of a knowledge of the mechanics involved in these processes of gametophytic selection, by which we may control the proportion of $(2n+1)$ types obtained in a sample of progeny.

ORDER OF FERTILIZATION IN OVARY

One may gain an idea of the extent to which the upper portion of a seed capsule is fertilized by the earliest arriving pollen tubes from an experiment conducted in 1922. The flowers of white plants were pollinated with pollen (20-60 grains) from purple plants, and repollinated after 24 hours by selfing. Thus the pollen tubes from the crossed pollen had a day's start over those of the selfed pollen, and must have reached the ovary first if they grew at all. The seedlings of the cross could easily be identified by their color, since purple is a dominant with respect to the recessive white gene. The seeds obtained from eight capsules were divided into three sections, representing upper, middle, and lower portions, and grown as separate pedigrees (table III). In the total, 92.2 per cent of the purple plants were found in the upper portion of the seed capsule, which represents the upper fourth of the total seed population, while only 6.7 per cent were found in the middle section, and only a single purple plant was obtained from seeds of the lower portion.

In another similar series of tests not tabulated here, a total of 987 plants from five seed capsules gave similar results. These five seed capsules were each divided into two parts, the upper halves containing an average of 94.5 per cent of the purple plants and the lower halves including only 5.5 per cent. Both experiments demonstrated conclusively that the early arriving pollen tubes enter the ovules situated near the top of the ovary.

WEDGE AS A SECONDARY OF COCKLEBUR

Wedge (Wd) is the only secondary of Cocklebur which has been recognized. In its breeding behavior, line 1A Wedge plants when

selfed have thrown 628 2n plants, 149 Wd, 8 Ck, its primary, and from one to two each of five other unrelated types. This breeding behavior indicates that Ck is its primary (2).

TABLE III

EARLY AND LATE ARRIVAL OF POLLEN TUBES IN OVARY DUE TO COUNTED POLLINATIONS, AND RE POLLINATIONS AFTER DEFINITE INTERVALS

No	SEEDS PLANTED	PLANTS RE- CORDED	REGION IN SEED CAPSULE					
			Upper		Middle		Lower	
			P	W	P	W	P	W
22-15.	102	89	17	72	1	145		...
	158	146						...
	155	148					o	148
22-16....	80	78	8	70	2	166		...
	178	168						...
	227	190					o	190
22-56....	122	111	38	73	1	256		...
	271	257						...
	275	253					o	253
22-62..	94	89	1	88	1	245		...
	266	246						...
	284	271					o	271
22-63 ..	101	96	6	90	o	193		...
	200	193						...
	146	136					o	136
22-64	177	160	5	155	o	159		...
	189	159						...
	179	171					1	170
22-65...	93	86	1	85	o	256		...
	260	256						...
	317	311					o	311
22-55....	87	74	7	67	1	150		...
	161	151						...
	167	140					o	140
Totals...		2979	83	700	6	1570	1	1619

No specific tests have been made of the pollen transmissibility of Ck from Wd pollen under conditions of restricted pollinations, but some data have been obtained from routine pollinations which show that Ck is present in significant proportions in the offspring of male

back-crosses of Wd. These data are included in table I, where an average of 2.7 per cent Ck was found in the offspring of the Wedge male back-crosses. These records from male back-crosses may therefore be taken as further evidence to indicate that Wedge is a secondary of Cocklebur.

Cytological evidence also indicates that Wedge is not a primary but a secondary (1), and its external and internal morphological features relate it to Ck (24).

POLLEN TUBE GROWTH FROM POLLEN OF WEDGE

Figs. 9-11 show pollen tube distribution curves of Wedge, and fig. 12 is that of a normal plant. A high proportion of the pollen of

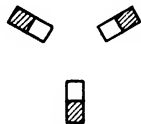


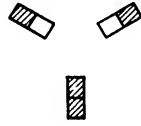
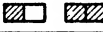

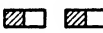

	TRISOME	DISJUNCTION OF TRISOME	PERFORMANCE OF GAMETOPHYTES	PLANTS OBTAINED IF UNITED WITH NORMAL EGGS
COCKLEBUR PRIMARY			Pollen tubes with slow growth	Cocklebur
			Normal pollen tubes	Normal
WEDGE SECONDARY			Pollen tubes swell and burst near stigma	Would produce Wedge
			Normal pollen tubes	Normal
			Pollen tubes with slow growth	Cocklebur
			Aborted pollen grains	?

FIG. 13

Wedge germinates. The pollen tubes with n chromosomes may be found in the longest group constituting the forward mode. A large group of short tubes, most of them swollen or burst, is found in the region close to the stigma; in some of the tests (figs. 9, 10) a group of normal appearing tubes is found forming a small mode between these two main groups.

Fig. 13 illustrates our scheme for the chromosomes of Wd and Ck. In Ck, which is a primary, are three identical chromosomes forming a trisome, hence there are only two types of microspores possible at reduction, those with n chromosomes and those with $(n+1)$. In Wd, however, which has one member of its trisome different from the other two, four classes of microspores are possible. We think of

the odd member of the trisome of Wd as made up of the shaded doubled half, of the Ck chromosome, and, if the assortment of chromosomes were entirely at random, there would form four classes of microspores: normal-producing n ; Wd-producing ($n+Wd$); Ck-producing ($n+Ck$); and ($n+Wd-Ck$), a class which is deficient. The n pollen tubes are easily identified (figs. 9-11); the ($n+Wd$) are likewise included in the large abnormal group near the stigma; the ($n+Ck$) group may be the small mode between these; and the ($n+Wd-Ck$) are represented by aborted pollen grains, not included in these tests. The assortment of chromosomes in the trisome of Wd at reduction is evidently not entirely at random, that is, 2:2:1:1, for the diagrams (figs. 10, 11) do not show half as many ($n+Ck$) pollen tubes as n and ($n+Wd$). Likewise the proportion of aborted pollen in Wd ranging from 6 to 10 per cent (4) is not as high as it should be if we assume random assortment of the chromosomes in the disjunction of this trisome. In fig. 11 the small ($n+Ck$) group is 8.5 per cent of all the normal pollen tubes which have passed or reached this point, and in fig. 10, where a similar group is found in a more retarded position, these represent 13 per cent of the pollen tubes which have passed or reached this distance. While the small groups of retarded normal-appearing pollen tubes in figs. 10 and 11 would seem to represent ($n+Ck$) tubes, it appears that this group may not always be identified, for it should be noted that in the test of fig. 9 (a companion test of fig. 10) no such second mode was observed.

If all the pollen tubes which have passed or reached the place of these small second modes (figs. 10, 11) would fertilize ovules giving rise to seeds (these groups resulting in Ck plants), there would be 73 Ck plants in a total of 786, or 9.3 per cent. This is about three and a half times in excess of the results shown in table I, where an average of 2.7 per cent Ck plants have been obtained from Wd male back-crosses in pollinations employing routine methods. However, the excess of Ck plants in table II, B over I (restricted pollinations versus routine pollinations) is in about this same proportion.

The fact that two Wedge plants were obtained in the back-crosses (table I) is rather surprising in view of the conditions shown in figs. 9-11, as well as in many other similar tests not included here. However, the proportion of Wd transmitted is only 0.3 per cent of the

total. The data in table I are not comparable with the graphs, since the latter are confined to line 1 plants and in table I the Wd plants that were back-crossed were heterozygous for other lines. The primary Pn heterozygous for certain lines when male back-crossed to these lines has produced as many as 11 per cent of Pn individuals. If only line 1 had been involved in these male back-crosses, no Pn individuals would have been expected in the offspring. The only male back-crosses of Wd involving merely line 1 in our records gave a total of 83 offspring, all normals.

It may be concluded that Wd is not transmitted through the pollen in line 1A. With the exception of the two Wd plants noted in table I when line 21 was involved, no instances of pollen transmission of Wd have been obtained.

Discussion

The successes secured in efforts to control the processes of gametophytic selection in the transmission of Ck through the pollen have been gratifying. It is possible to obtain conditions in which none of the Ck is transmitted, or to vary the conditions so that half or more than half of the seeds selected for planting will produce Ck plants. The method for obtaining no Ck plants through the pollen is to pollinate with much pollen, and, if possible, to excise the styles after the first group of pollen tubes have entered the ovary. If styles are not excised, only the seeds from the upper half of the seed capsules must be planted. The method for obtaining a maximum of Ck transmission is to pollinate sparingly (with 400–500 pollen grains) and to plant only the seeds taken from the lower half or third of moderate-sized seed capsules. Small seed capsules are often the result of severe interovular selection, in which the ovules containing $(2n+1)$ zygotes suffer a higher mortality than those containing $2n$ zygotes.

All the experiments have confirmed our interpretation regarding the identity of the groups of pollen tubes visible in the microscopic preparations. We may now predict from similar tests of other $(2n+1)$ plants, where it may be feasible to make male back-crosses using restricted pollinations with the expectation of obtaining transmission of the extra chromosome.

Since several methods have been combined in this study of the

pollen transmissibility of Ck and Wd, it may be of interest to enumerate briefly these methods as used by various investigators, where pollen tube growth conditions were involved.

RESTRICTED VERSUS ABUNDANT POLLINATION

CORRENS (11) used small versus large amounts of pollen in pollinations of the dioecious plant *Melandrium*. He obtained an increase in the proportions of staminate plants when small amounts of pollen were employed and a decrease with large amounts. His explanation, based on the theory that pollen tubes carrying the staminate determining gametes grow slower than pollen tubes transmitting the pistillate determining gametes, was confirmed by many subsequent experiments (12, 13).

In an investigation on the number of pollen grains necessary to induce seed setting in *Mirabilis*, NAUDIN (23) was the first to make counted pollinations. GOODSPEED and DAVIDSON (18) counted the pollen grains and determined the minimum number necessary to obtain seeds in *Nicotiana langsdorffii* var. *grandiflora*, and SNOW (26) used counted pollinations in a genetic study of *Matthiola*.

We have employed restricted pollinations since 1921 in a study of the pollen transmissibility of Globes (14, 15) by a method of counting the pollen grains. The results, which have not been published in detail, have demonstrated that the pollen tube growth phenomena involved in the transmission of Globes may be modified experimentally by the abundance of pollen on the stigma, but in *Datura* there is also a crowding effect among the ovules. As a result of this process of interovular selection, the proportions of $(2n+1)$ plants which survive are further modified.

EXCISION OF STYLES

Excision of styles at various intervals after pollination was a method employed by CORRENS in *Melandrium* (13). HERIBERT-NILSSON (19) employed the method of cutting off styles in a study of "certation" in *Oenothera lamarkiana*. He compared the time required for obtaining fertilization in flowers pollinated with their own pollen with those on other flowers of the same plant pollinated with the pollen of the mutant form *gigas*, and found that the pollen tubes of the

last named form require a slightly longer period of growth to accomplish fertilization before excision than the pollen tubes from selfing. In a later investigation (20) he discovered by properly timed style-excisions that from pollen heterozygous for *rubrinervis* there are many more pollen tubes bearing the genes for *rubrinervis* among those which arrive at the ovary during the earliest hours of fertilization than one or two hours later.

DAVIS (16), using the cross *Oenothera lamarckiana* × *nanella-brevistylis*, excised the styles at various intervals after pollination and obtained in a rather limited population (total of 100 plants) ratios of 1:5 when the styles were cut off 23 hours after pollination, and a ratio of 1:2.8 when they were cut off 24 hours after pollination. Likewise SIRKS (25) obtained differences in the ratios of *Datura* in crosses involving the segregation of purple-armed and white-inermis in male back-crosses, by cutting off the styles at various intervals after pollination.

PROPORTIONS FOUND IN UPPER AND LOWER HALVES OF SEED CAPSULES

CORRENS (12) proved experimentally that the ovules in the upper part of the ovary of *Melandrium* are entered by the pollen tubes which arrive early, and introduced the methods of dividing the pedigrees into upper and lower portions. He found the proportions of staminate and pistillate plants derived from the seeds of these regions of the ovary consistent with his theory that the staminate determining pollen tubes are slower than the pistillate determining pollen tubes.

Similar methods have been employed by JONES (21), BRINK (5), and other breeders of maize, using styles left long, compared with styles cut short, and counting the proportions in upper and lower parts of the ears separately, etc. These methods are fully discussed by JONES, and while some of the factors involved seem to be similar to ours, they are not further cited and discussed here, because the conditions are not entirely comparable with the present problem. The ear of maize is an inflorescence of many one-ovuled pistils, and we are concerned with single flowers having compound pistils with a common style and stigma and many ovules.

POLLEN TUBE DISTRIBUTIONS DURING GROWTH

EAST and PARK (17) made the first attempts to analyze a pollen tube population by the use of histological methods, using serial paraffin sections, stained and reconstructed so that the distribution of the tubes could be plotted. We have adopted a similar method, following a more rapid and accurate special technique of dissection, and have plotted pollen tube distribution curves in a number of investigations. It has seemed advisable, therefore, to test the validity of this rapid technique in the study of pollen tube growth, by employing this new method together with as many other methods as possible in an attack on an individual problem.

The experience of cotton breeders in excising styles has not given support to a theory based on differential growth rate of pollen tubes. KEARNEY and HARRISON (22) failed to obtain significant differences in the proportion of hybrids and selfs when they employed pollen mixtures of Upland and Pima cotton, or when they used excision of styles; they also failed to find differences in the proportions between the upper and lower halves of seed capsules, although their experience indicated that there must be some kind of inequality in the ability to accomplish fertilization by the two kinds of pollen.

In the face of the results of KEARNEY and HARRISON, it would seem to be desirable to have every method used in such investigations thoroughly tested. In this study of pollen transmissibility we have shown that there is complete agreement in our results from the employment of four of these methods of investigation, and there can be no question about the fact that the pollen tubes carrying the extra Ck chromosome have a slower rate of growth than normal ones.

In the study of the pollen of heterozygous plants, our pollen tube counting method has a distinct advantage in enabling us to distinguish between the following types of pollen tube growth conditions which may result in the complete or partial failure of pollen transmission when gametophytic selection is involved: (1) complete failure of half of the pollen to germinate on the stigma, as in the case of polycarpic (10); (2) delayed germination of half of the pollen, resulting in bimodal distributions; (3) differential growth rate between two classes of pollen tubes also resulting in bimodal distributions, the last two conditions being distinguishable by comparing

pollen tube growth tests made after different intervals of time; (4) complete elimination of half of the pollen tube population through bursting as in Wd, and the question of whether or not this is preceded by a delay in germination; (5) partial elimination of half of the pollen tube population through bursting of pollen tubes, as we have found in the tricarpel race of *Datura* (8).

Summary

1. Pollen tube distributions were plotted from 12-, 16-, and 18-hour tests, using the pollen of the primary ($2n+1$) Cocklebur germinated and grown on the pistils of $2n$ plants. These tests were compared with similar tests of pollen tube growth of normal plants in line 1A.

2. Further evidence is given from the results of ordinary male back-crosses of Wedge to four lines, indicating that the latter is a secondary ($2n+1$) type related to Cocklebur. Pollen tube distributions obtained from the pollen of Wedge are also plotted.

3. The pollen tube distribution curves indicate that Cocklebur may be transmitted through the pollen under conditions in which the processes of gametophytic selection are controlled experimentally. Likewise, the distribution curves indicate that Wedge may not be transmitted through the pollen in line 1A.

4. The correctness of the interpretations gained from pollen tube distribution curves was tested genetically in the case of Cocklebur, by combining three other experimental methods: the effects of restricted pollinations; the effects of excision of styles; and the separation of the seeds in the lower half of seed capsules from those in the upper half. Results from all experimental methods confirmed the interpretations gained from a study of pollen tube distribution curves.

5. The various methods which have been used by investigators at different times in a study of the effects of gametophytic selection are briefly discussed and compared.

6. The pollen tube distribution curves obtained from suitable tests enable us to recognize processes of gametophytic selection, and have the further advantage of enabling us to distinguish between at least five different conditions, all of which may lead to the complete

or partial elimination of some of the classes of male gametophytes, resulting in modified ratios in the progeny.

7. The processes of gametophytic selection involved in the pollen transmission of Cocklebur may be controlled through a wide range. A threefold increase may be obtained by restricted pollinations, and a further increase, up to 65 per cent Cocklebur plants, may be obtained in a progeny by selecting the seeds to be planted from the lower parts of seed capsules, while complete non-transmission of Cocklebur may be obtained by properly timed excisions of styles.

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NEW OR OTHERWISE NOTEWORTHY COMPOSITAE. V
CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 408

EARL EDWARD SHERFF

(WITH PLATES IV, V)

COREOPSIS BORIANIANA Schz. Bip. *ex* Schweinf., Verhandl. Zool.-Bot. Ges. Wien 18:684. 1868; *C. borianiana* var. *cannabina* Schz. Bip. *loc. cit.*; *C. guineensis* O. & H. in Oliver Fl. Trop. Afr. 3:390. 1877; *C. chrysopterocarpa* Chiov., Ann. Bot. Roma 9:75. 1911.

SCHULTZ BIPONTINUS based his *C. borianiana* upon (1) *Boriani* 42, in the Fesoglu, 1839 and (2) *Schweinfurth* 439, Matamma, Oct. 9, 1865. He based his var. *cannabina* upon a larger specimen by *Schweinfurth*, no. 585, also collected at Matamma in the middle of October, 1865. The species proper, as represented by the *Boriani* specimen (I have seen SCHULTZ BIPONTINUS' type fragment at Paris), has leaf segments 3-6 mm. wide, while the var. *cannabina* has leaf segments 0.8-2.2 cm. wide. These distinctions appear to rest, however, upon different stages of development. Thus, for example, a specimen of *Schweinfurth* 439 in SCHULTZ BIPONTINUS' own herbarium (in Herb. Par.) has, above, the narrow leaf segments described for the species, and, below, one leaf with the broader segments described for the variety and matching the leaves of *Schweinfurth* 585 (Herb. Berl.). In fact, SCHULTZ BIPONTINUS himself had labeled this specimen var. *cannabina*.

With these considerations in mind, I have reduced the var. *cannabina* to synonymy with *C. borianiana* proper.

OLIVER and HIERN (*in* Oliver *loc. cit.*) based their *Coreopsis guineensis* primarily upon a plant by *C. Barter*, collected at Nupe, Nigeria (Herb. Kew). The *Barter* plant is purely *C. borianiana*, as are also many other plants secured by various collectors more recently and referred in herbaria to *C. guineensis*.

Some time ago I was permitted, through the kindness of Dr. GIOVANNI NEGRI of Florence, Italy, to examine the type of *C. chrysopterocarpa* Chiov. (*Pappi* 7733, alt. 1000 m., Baza-Cunama-Barentù,

Eritrea, Nov. 28, 1908, in Herb. R. Inst. Bot. Florence). It proved to be identical with *C. guineensis* O. & H. and hence with *C. borianiana*.

Specimens examined: *C. Barter* 933, Nigeria, 1857-1859 (Herb. Berl.; Herb. Gray); *Boriani* 42, in the Fesoglu, northeastern Africa, 1839;¹ *Aug. Chevalier* 2083, Koulitsoro, Sudan, 1899 (Herb. Berl.; Herb. Bruss.); *idem* 9633, Massenya (Massenia), Bagirmi (Baguirmi), Sudan, 1902-1904 (Herb. Berl.); *Dr. Kersting* 14, alt. 350 m., Trógode, Kerkri, Togo, Oct. 28, 1897 (Herb. Berl.); *idem* 422, alt. 400 m., Sokodé-Basari, Togo, October, 1901 (Herb. Berl.); *Lécard* 290, Sénégal (Herb. Bruss.); *C. Ledermann* 5243, growing 1-1.5 m. high, alt. 380 m., between Tschamba and Doreba, Kamerun, Sept. 18, 1909 (Herb. Berl., 2 sheets); *A. Pappi* 7733, alt. 1000 m., Baza-Cunama-Barentù, Eritrea, Nov. 28, 1908 (Herb. Flor.; type of *Coreopsis chrysopterocarpa* Chiov.); *Dr. T. Pfund*, Dar-Fur, 1876 (Herb. Berl.); *idem* 336, Takari on the Rahad, Kordofan, July, 1875 (Herb. Berl., 2 sheets); *Dr. Rowland*, common in savannahs, western Lagos, 1893 (Herb. Berl., 2 sheets); *F. Schroeder* 74, alt. 350 m., Sokodé Farm, Togo, Oct. 19, 1900 (Herb. Berl., 2 sheets); *Dr. G. Schweinfurth* (Flora of Gallabat no. 439), Matamma, Gallabat (Galabat) region, northwestern Abyssinia, middle of October, 1865 (Herb. Schz. Bip. in Herb. Par.); *idem* (Flora of Gallabat no. 585), *eodem loco et tempore* (Herb. Berl.; type material of var. *cannabina* Schz. Bip.).

COREOPSIS BORIANIANA *multiplex* var. nov.—A specie involucri bracteis exterioribus biserialibus 18-22, plerumque 1.4-1.8 cm. longis quam interioribus saepe longioribus differt.

Specimens examined: "*Graf. Zech.* 168," Kete Kratschy, Togo, Oct. 21, 1898 (Herb. Berl.; type).

COREOPSIS PACHYLOMA O. & H. in Oliver Fl. Trop. Afr. 3:391. 1877; *Coreopsis involucreta* Schz. Bip. in Walpers Repert. 6:163. 1846 (non Nutt.); *Verbesina involucreta* (Schz. Bip.) A. Rich. Tent. Fl. Abyssin. 1:409. 1847; *Coreopsis callosa* Schz. Bip. in Schweinf. et Aschers. Enum. 284.

SCHULTZ BIPONTINUS definitely published his *Coreopsis involu-*

¹ The type was cited for the Herbarium of the Museum of Vienna, and doubtless I examined it there some years ago. I have had at hand more recently, however, the type fragment from SCHULTZ BIPONTINUS' herbarium (in Herb. Par.).

crata in 1846 with a valid description. Later, to avoid conflict with NUTTALL'S *C. involucrata*, he renamed his own species *C. callosa*.

My recent study of the types and other specimens of *Coreopsis pachyloma* O. & H. and of *C. involucrata* Schz. Bip. showed them to be identical. OLIVER and HIERN clearly were unfamiliar with the status of *C. involucrata* Schz. Bip. and its equivalent, *C. callosa* Schz. Bip., for they published these two names (Oliver Fl. Trop. Afr. 3:392. 1877) as of "unknown species of *Coreopsis*" and treated them both as *nomina nuda*.

In recent decades *Coreopsis involucrata* Nutt., a species of North America, has become generally accepted as belonging in *Bidens*. In that genus it is *B. involucrata* (Nutt.) Britt. under the Vienna Rules, or *B. polylepis* Blake under the so-called American Rules. Thus *Coreopsis involucrata* Schz. Bip. is left as the next name for the species in *Coreopsis* that was later named *C. pachyloma* and, if the rules as to synonymy in the Vienna Code (versus the American) were to be followed, would take precedence over *C. pachyloma* O. & H.

Specimens examined: *Rüppell*, between Temben and Siemen, Abyssinia, June or July, 1832 (Herb. Par.; type of *C. involucrata* Schz. Bip.); *Wilhelm Schimper* (*iter abyssin. sect. V*) 15, Abyssinia, 1853 (Herb. Berl.; Herb. Deless.; Herb. Par.); *idem* (*iter abyssin. sect. V*) 933 *pro parte*, Abyssinia (Herb. Par.); *Dr. Steudner*, Gaffat near Debra Tabor, Abyssinia, May 10, 1862 (Herb. Berl.).

COREOPSIS PACHYLOMA inanis var. nov.—A specie achaeniis etiam primo exaristatis differt.²

Specimens examined: *Wilhelm Schimper*, no. 933 *pro parte*, Abyssinia, *commun.* 1853 (Par.).

COREOPSIS OLIGOFLORA robusta var. nov.³—A specie involucri bracteis exterioribus late oblongis vel ovatis saepius 2.5–4 mm. latis apicaliter obtusissimis differt.

Specimens examined: *Büttner* 408 (Herb. Berl.; type); *P. Pogge* 1292, Mukenge tract (*campine*), western Africa, Apr. 13, 1882 (Herb. Berl.).

² The American Code is followed in this instance. As already implied, the Vienna Code would require *C. involucrata* var. *inanis* for the name here.

³ The name *robusta* was used for this variety on a herbarium label by Dr. OTTO HOFFMANN, in connection with KLATT'S later (and philologically better) name *C. oligantha*. I do not seem, however, to have noticed the name in print.

COREOPSIS PRESTINARIAEFORMIS *incisa* var. nov.; *C. heterocarpa* Chiov., Ann. Bot. Roma 9:75. 1911 (*pro parva parte*).—A specie foliis acriter et perspicue bipinnatisectis segmentis principalibus oblongo-lanceolatis differt.

Specimens examined: *Dr. Emilio Chiovenda* 1090, very common, Gondar, Province of Amhara, Abyssinia, Jul. 26, 1909 (Herb. Flor.); *Dr. Ellenbeck (Exped. Baron von Erlanger)* 1436, growing 1 m. high, alt. 2600 m., Djafa, Arussi Galla, Gallaland, Jul. 21, 1900 (Herb. Berl.; type).

In July–September, 1909, CHIOVENDA collected in the Province of Amhara, Abyssinia, seven plants which he assumed to be the same. Of these I have examined six, and find five (nos. 970, 1148, 1869, 1953, and 1954) to be *C. prestinariaeformis* Vatke.⁴ A sixth one (no. 1090) has noticeably bipinnatisect foliage, such as is found commonly in *C. prestinaria* Schz. Bip., and easily merits varietal distinction.

Unfortunately, CHIOVENDA seems to have been unfamiliar with *C. prestinariaeformis* Vatke (the types of which are at Berlin). He founded his *C. heterocarpa* upon these seven specimens. From alphabetical and numerical considerations his no. 1090 (my var. *incisa*) was cited first but his description clearly was based principally upon the other specimens, that is, those of *C. prestinariaeformis* proper. Hence it is impossible of course to utilize here the name *heterocarpa*. I have chosen, therefore, the entirely new name *incisa*.

Bidens chaetodonta nom. nov.; *Coreopsis abyssinica* Schz. Bip. in Walpers Repert. 6:163. 1846; *Verbesina abyssinica* (Schz. Bip.) A. Rich. Tent. Fl. Abyssin. 1:409. 1847; *Prestinaria (Steppia) abyssinica* Schz. Bip. in Herb. Schimp. Abyssin. sect. I, n. 332 ex O. & H. in Oliver Fl. Trop. Afr. 3:389. 1877.

Specimens examined: *Dr. Emilio Chiovenda* 3151, Province of Amhara, Abyssinia, Dec. 7, 1909 (Herb. Flor., 2 sheets); *Quartin Dillon*, Abyssinia (Herb. Par.); *A. Petit*, Abyssinia (Herb. Berl.; Herb. Par.); *idem*, Chiré, Abyssinia (Herb. Berl.); *Wilhelm Schimper* 332, in upper region, northern side of Mt. Scholoda, Abyssinia, Oct. 3, 1837 (Herb. Berl.; Herb. Par., 5 sheets; type material).

⁴ The type specimens of which (Herb. Berl.) have rather immature capitula and fairly slender achenial aristae (cf. O. & H. in Oliver Fl. Trop. Afr. 3:389. 1877: "filiform acute aristae"). *Chiovenda's* specimens are taller, more robust, and have many mature achenes with the characteristically lanceolate-subulate aristae such as are found also in *C. macrantha* Schz. Bip., etc.

BIDENS CHAETODONTA var. **glabrior** (O. & H. in Oliv.) comb. nov.;
Coreopsis abyssinica var. *glabrior* O. & H. in Oliver Fl. Trop. Afr.
3:389. 1877.

Specimens examined: *Dr. Ellenbeck* 1566, alt. above 2000 m., Akaki, South Schoa, Abyssinia, Aug. 13, 1900 (Herb. Berl.); *idem* 1592, alt. about 2300 m., Adis Abeba, Schoa, Oct. 3, 1900 (Herb. Berl.); *idem* 1614, alt. 2300 m., Akaki, Dec. 26, 1900 (Herb. Berl.); *A. Pappi* 5205, alt. about 2610 m., Mt. Zagher, Eritrea, May 20, 1902 (Herb. Flor.); *A. Petit*, Chiré, Abyssinia (Kew; type); *idem* 138, *eodem loco*, August, 1840 (Herb. Par.); *idem* 587, *eodem loco* (Herb. Par.).

Bidens dielsii nom. nov.;⁵ *Coreopsis chrysantha* var. *simplicifolia* Vatke, Linnaea 5:500. 1875; *C. simplicifolia* (Vatke) Engler Hochgeb. Fl. Trop. Afr. 435. 1892.

Specimens examined: *Oscar Neumann* 135, alt. 2800 m., mountain meadows, Gardulla, Gallaland, Jan. 14, 1901 (Herb. Berl.); *Dr. Rugazzi* 1311, Dallo Scioa, Tropical East Africa, 1887 (Herb. Flor., 2 sheets); *Wilhelm Schimper*, alt. 7000 ft., on mountains at Dewari, Abyssinia, Oct. 2, 1863 (Herb. Berl.; 2 type sheets).

It is difficult when studying the type collections to understand why VATKE referred this plant to a variety of his *Coreopsis chrysantha*, even though they were both collected by *Schimper* and on the same day. OLIVER and HIERN (*loc. cit.*) stated: "This variety of VATKE appears to constitute a distinct species. . . ." Later, ENGLER very properly elevated it to specific rank. The species is better placed, however, in *Bidens*.

Bidens vatkei nom. nov.; *Coreopsis chrysantha* Vatke, Linnaea 39:499. 1875 (*non* L. Sp. Pl. edit. II:1282. 1763).

Specimens examined: *Dr. Emilio Chiovenda* 1699, Gondar, Dembià, Province of Amhara, Abyssinia, Aug. 28, 1909 (Herb. Flor.); *forma foliis majoribus, foliolis latioribus terminali usque ad 3.5 cm. lato*; *idem* 1992, near Asosò, Dembià, Sept. 13, 1909 (Herb. Flor.); *Wilhelm Schimper*, alt. 7000 ft., Valley of Repp River, Dewari, Abyssinia, Oct. 2, 1863 (Herb. Berl., 2 sheets of type material).

⁵ The type material of this and many other species was lent to me for study several years ago through the kind permission of Dr. LUDWIG DIELS, Director of the Berlin Botanical Garden. Because of the existence already of *Bidens simplicifolia* Wright, however, the adoption here of a new trivial name becomes compulsory. In this connection it is a pleasure to commemorate Dr. DIELS' name and thus help to repay in small part the great debt that the science of botany owes him for this invaluable aid.

Bidens rueppellii (Schz. Bip.) comb. nov.; *Coreopsis rueppellii* Schz. Bip., Walpers Repert. 6:163. 1846; *Verbesina rueppellii* A. Rich. Tent. Fl. Abyssin. 1:410. 1847; *V. rueppellii* (Schz. Bip.) A. Rich. ex Vatke Linnaea 39:499. 1875.

Specimens examined: *Dr. Emilio Chiovenda* 2930, Debarek, Semien, Province of Amhara, Abyssinia, Nov. 29, 1909 (Herb. Flor., 2 sheets); *idem* 2906, Darà, Uogherà, Province of Amhara, Nov. 28, 1909 (Herb. Flor.); *Rueppell*, Siemen, Abyssinia, August or September, 1832 (type, Herb. Par.; a mere fragment but decisively distinctive); *Wilhelm Schimper*, Abyssinia (Herb. Par.); *idem* 706B, Abyssinia, 1854 (Herb. Deless.; Herb. Par.).

A hitherto but little known species. VATKE (*loc. cit.*) doubtfully referred it to his *Coreopsis chrysantha* (my *Bidens vatkei*) while OLIVER and HIERN (Oliver Fl. Trop. Afr. 3:388. 1877) stated that it was unknown to them. The achenes are black, exalate, and match those of many other species of *Bidens*.

BIDENS BITERNATA var. *glabrata* (Vatke) comb. nov.; *B. abyssinica* var. *glabrata* Vatke, Linnaea 39:500. 1875.

VATKE's variety *glabrata* was described as having a glabrous stem and was based upon a plant by *Wilhelm Schimper*, no. 105, Gaha Meda near Dschadscha, Abyssinia, 1854. VATKE's variety appears to have been completely overlooked by subsequent workers. It is the glabrous-stemmed variety of the plant which was named *Bidens abyssinica* by SCHULTZ BIPONTINUS and later renamed *B. chinensis* var. *abyssinica* O. E. Schz., also *B. biternata* var. *abyssinica* Sherff. Under the Vienna Code the name *glabrata* must be taken up for the varietal rank.⁶

The hairy-stemmed form of this variety has not been generally distinguished by me in my herbarium determinations thus far, but is the form described originally as *Bidens abyssinica* Schz. Bip. (Walpers Repert. 6:167. 1846) and later as *B. quadriseta* Hochst. (*in* Herb. Schimp. Abyssin. ex O. & H. *in* Oliver Fl. Trop. Afr. 3:393. 1877). As the hairy-stemmed plants appear to be fairly constant as well as of strikingly different appearance, they may be set off under the var. *glabra* as f. *abyssinica* (Schz. Bip.) comb. nov.

⁶ Certain earlier varietal names (*B. abyssinica* var. *quadriaristata* Hochst. ex Schweinf. Beitr. Fl. Aethiop. 142. 1867; var. *incisifolia* Hochst. *in herb.*, etc.) were not validly published.

Bidens microphylla nom. nov.; *Coreopsis pulchella* O. Hoffm., Engler Bot. Jahrb. 38:204. 1906; Engler Pflanzenw. Afr. 1: 155. tab. 124. 1910.

Specimens examined: *Dr. Ellenbeck* 1370, alt. 3000 m., on rocks, Abulkasin, Arussi Galla, southern Abyssinia, Jul. 16, 1900 (Herb. Berl.; type).

Bidens setigera (Schz. Bip.) comb. nov.; *Coreopsis setigera* Schz. Bip., Walpers Repert. 6:163. 1846; *Chrysanthellum (Microlecan) abyssinicum* Schz. Bip., loc. cit. 171; *Verbesina lineata* A. Rich. Tent. Fl. Abyssin. 1:410. 1847; *Coreopsis abyssinica* var. *bipinnato-partita* Chiov. ex Pirotta Fl. Colon. Eritrea 1:185. 1904.

Specimens examined: *Dr. Emilio Chiovenda* 2051, Gondar, Province of Amhara, Abyssinia, Sept. 13, 1909 (Herb. Flor.); *idem* 2699, Province of Amhara, Oct. 27, 1909 (Herb. Flor.); *idem* 2750, Gondar (Herb. Flor.); *idem* 3253, Division of Tigré, Abyssinia, Dec. 11, 1909 (Herb. Flor.); *A. Pappi* 559, alt. about 2000 m., Adi Quala, Saraë, Eritrea, Oct. 23, 1902 (Herb. Flor.); *idem* 3871, Eritrea, Mar. 2-10, 1902 (Herb. Flor.); *idem* 4101, alt. about 600 m., Valle Damas, Oculé Cusai, Eritrea, Apr. 14, 1893 (Herb. Flor.); *idem* 4209, Dongollo near Ghinda, Eritrea, Mar. 12, 1902 (Herb. Flor.); *Dr. A. Ragazzi* 3360/3860, Scioa, Tropical East Africa, Oct. 10, 1886 (Herb. Flor.); *Rüppell*, Abyssinia, 1831-1832 (Par.; type); *Wilhelm Schimper (iter abyssin. sect II)* 766, along edges of groves in valleys near Adoa, Abyssinia, Nov. 12, 1838 (Herb. Par.; type of *Chrysanthellum abyssinicum* Schz. Bip.); *Sing Scotti*, Ghinda, Eritrea, 1893 (Herb. Flor.).

Bidens magnifolia nom. nov.; *Coreopsis frondosa* O. Hoffm., Engler Pflanzenw. Ost-Afr. C.414. 1895. Pl. IV.

Specimens examined: *Albers* 204, growing 2-3 m. high at alt. 1600 m., Kwai, German East Africa, Oct. 22, 1899 (Herb. Berl.); *Karl Braun* 671, growing over 1 m. high, Amani, German East Africa, May 12, 1905 (Herb. Berl., 2 sheets); *idem* 2682, Lutindi, East Usambara, German East Africa, Aug. 15, 1909 (Herb. Berl.); *Dr. J. Buchwald* 263, growing 2.5 m. high, Usambara, Dec. 20, 1925 (Herb. Berl.); *C. Holst* 2252, growing 2-2.5 m. high, Kwa Kiniarri, Nderema, Tropical East Africa, Feb. 23, 1893 (Herb. Berl.; type); *idem* 9149a, alt. 1600 m., high forest clearings, Kwa Mstuzi, Tropical East Africa, Aug. 17, 1893 (Herb. Berl.); *Dr. Houy (Exped. Hans Meyer)* 1190, growing 1-2 m. high, Nsogiro Mountains, Ussagara, District of Kilossa, Morogoro, German East Africa, November-December, 1911 (Herb. Berl.); *Münzner (Exped. Capt. Fromm)* 35, growing 2 m. high along brook, Langenburg, German East Africa, Jul. 30, 1908 (Herb. Berl.); *Preuss* 689, Buea, Kamerun, 1891 (Herb. Berl.); *Dr. Franz Ludwig Stuhlmann* 8912, alt. 1400 m., German East Africa, Oct. 20, 1894 (Herb. Berl.); *idem* 9265, German East Africa, Nov. 20, 1894 (Berl.);

Warnecke 63, growing 1.5 m. high, alt. 700-900 m., Amani, German East Africa (Herb. Berl.); Albrecht Zimmermann 63, alt. 900 m., Amani, December, 1902 (Herb. Berl., 2 sheets).

Bidens ternata (Chiov.) comb. nov.; *Coreopsis ternata* Chiov., Ann. Bot. Roma 9:74. 1911.

Specimens examined: Dr. Emilio Chiovenda 2581, very rare. Valley of Scintà above Asosò, Dembià, Abyssinia, Oct. 17, 1909 (Herb. Flor.; type).

Bidens rotata sp. nov.; *Coreopsis buchingeri* Schz. Bip. ex Schweinf. et Aschers. Enum. 284. (*nomen*).—Herba perennis, erecta, 6-10 dm. alta, supra ramosa, caule infra fere glabra supra hispida. Folia opposita subtenuiter petiolata petiolis planis hispidociliatis basaliter in poculum circ. 1 mm. altum connatis usque ad 2 cm. longis, petiolo adjecto saltem 4-7.5 cm. longa, tripartita foliolis membranaceis apicaliter subacuminatis ad medium usque ad 2.7 cm. latis nunc ovatis nunc lanceolatis supra viridibus ac subglabris infra pallidis ac papillato-hispidis margine ciliatis ac acerrime serratis dentibus mucronato-setigeris. Capitula tenuiter pedunculata pedunculis hispidis usque ad 18 cm. longis, radiata, pansa ad anthesin circ. 3.5 cm. lata et 1-1.2 cm. alta. Involucri bractee exteriores 10-13, lineari-subspathulatae, pallidae, dorso subglabrae vel aegre adpresso-hispidae, margine spinuloso-ciliatae, apice nitido callosomucronatae, 1-1.6 cm. longae et 1-2 mm. latae saepe interiores ovato-lanceolatas dorsaliter hispidas apicaliter ciliatas paulo superantes. Flores ligulati 12, aurei, ligula anguste oblongo-elliptici, apice 2-3-denticulati, circ. 1.5 cm. longi et circ. 4-5 mm. lati; tubulosi superne aurantiaci, sicci ad medium plus minusve tumidi vel fracti vel articulati. Achaenia plana, lineari-oblonga, nigra, unaquaque facierum circ. 4-sulcata et superne adpresso-setosa setis erectis, marginibus erecto-setosa, margines versus tenuia sed non vere alata, corpore 4-6 mm. alta et 1-1.2 mm. lata, apice erecte setosa ac biaristata aristas tenuibus stramineis sursum hispidis circ. 2 mm. longis.

Wilhelm Schimper (itin. abyssin. sect. V, n. 706a, edit. Buchinger ann. 1854) in Abyssinia (type in Herb. Paris).

The type is in SCHULTZ BIPONTINUS' private herbarium at Paris. By him it had been given the name *Coreopsis buchingeri*, which name has appeared occasionally in literature but without description. The

closest ally is *Bidens rueppellii*. *B. rotata* appears to differ, however, in having more glabrous stems, different foliage, larger involucre bracts, and larger achenes. The slender rays, regularly 12, offer a fanciful resemblance to the spokes of a wheel, whence the trivial name.

BIDENS STEPPIA leptocarpa var. nov.—Herba 1-1.5 m. alta. Capitula pansa ad anthesin 5-6.5 cm. lata; bracteis exterioribus 8-10, circ. 9-14 mm. longis et 1.2-2 mm. latis, quam interioribus oblongo-ovatis paulo longioribus; floribus ligulatis plerumque 8, circ. 2.5-3 cm. longis. Achaenia anguste oblongo-linearum, plana, nigra, unaquaque facierum circ. 8-sulculata, marginibus apiceque erecto-ciliata, faciebus praecipue supra plus minusve erecto-setosa, exalata, corpore 7-10.5 mm. longa et 1.1-1.3 mm. lata, biaristata aristis erectis stramineis tenuibus sursum hispidis circ. 2 mm. longis.

Specimens examined: *Braun* 5505, growing 1 m. high, Niamutukusja Nsira, District of Bukoba, German East Africa, June 15, 1913 (Herb. Berl.); *Ad. Stolz* 729, growing 1.5 m. high at alt. 1350 m., Langenburg, German East Africa, May 26, 1911 (Herb. Berl., type; Herb. Deless., 2 cotype sheets); *Dr. Franz Ludwig Stuhlmann* (*Exped. Emin Pascha*) 4156, Nuansa, Tropical East Africa, May 20, 1892 (Herb. Berl.).

BIDENS STEPPIA var. **ambacensis** (Hiern) comb. nov.; *Coreopsis ambacensis* Hiern Cat. Welw. Afr. Pl. 3:586. 1898; *Bidens ambacensis* (Hiern) Sherff, BOT. GAZ. 59:309. 1915.—The type was collected by *Welwitsch*, no. 3272 *pro parte*, in marshy places on the left bank of the Caringa River, Ambaca, western Africa, June, 1855.⁷ Six of the eight heads present were in fruit. The achenes are 5-7 mm. long and 0.8-0.9 mm. wide. Aristae are lacking, but in their place are two short teeth. The general habit of the plants is that of the broader-achened *Bidens steppia*, a species which *Welwitsch* likewise collected (no. 3531, Herb. Brit. Mus.; Herb. Kew: no. 3532, Herb. Brit.; Herb. Kew; Herb. Par.).

On transferring *Coreopsis ambacensis* to *Bidens* in 1915, I was unable to refer it to *Bidens steppia*, because of *B. steppia*'s normally larger achenes and the fact that transitional forms had not been studied. The finding of the foregoing var. *leptocarpa*, however, also

⁷ Type in Herb. Brit. Mus. Nat. Hist. A specimen at Kew bears the same number but is *Bidens grandis* Sherff, a very different species.

of several somewhat intermediate forms (as to achenial measurements) shows that *Coreopsis ambacensis*' claim to distinction is at best of only a varietal nature and rests upon the narrower, exaristate achenes.

BIDENS HOLSTII var. **rupestris** comb. nov.; *B. rupestris* Sherff, BOT. GAZ. 76:144. 1923.—A comparison of the type of *B. rupestris* with the type and other authentic specimens of *B. holstii* shows that the only essential difference is in the achenes. In *B. holstii* these are normally exaristate or at best weakly aristate with upwardly directed bristles. In *B. rupestris* they are definitely aristate with usually 1-3 retrorse barbs.

Bidens cirsioides sp. nov. (Pl. V).—Herba elata, verisimiliter perennis, glaberrima, pallida, 8-15 dm. alta, caule subtereti glaucescenti valde ramoso ramis elongatis plus minusve simplicibus. Folia petiolata petiolis planis anguste alatis basaliter dilatatis 0.5-2 cm. longis, petiolo adjecto usque ad 1.5 dm. longa, pinnatim 3 (-5)-partita, foliolis oblonge linearibus vel lineari-lanceolatis crassiusculis nunc perspicue acriterque dentatis dentibus elongatis saepe inflexis et saepe setigeris nunc setis pro dentibus munitis infra albescentibus margine saepe revolutis terminali usque ad 12 cm. longo et 2.5 cm. lato lateralibus minoribus. Capitula numerosa corymboso-paniculata, tenuiter pedicellata pedicellis hispidis usque ad 1 dm. longis, radiata, pansa ad anthesin 4-5 cm. lata et 8-11 mm. alta. Involucri bractee exteriores 16-24, subbiseriales, lineares, glaberrimae, apicem versus angustatae apice acriter calloso-mucronatae 8-10 mm. longae et 0.5-1 mm. latae; interiores late lanceolatae dorso ventreque apicaliter pubescentes alibi glabrae saepe paulo breviores. Flores ligulati plerumque 12, flavi, ligula lineari elliptici, paucistriati, apice subacuto vix denticulati, 1.8-2.3 cm. longi et circ. 3-4 mm. lati. Achaenia plana, lineari-oblonga, nigra, exalata, unaquaque facierum circ. 6-sulcata, faciebus marginibus valde erecto-setosa, corpore circ. 5 mm. longa et circ. 1-1.1 mm. lata, apice erecto-setosa ac biaristata aristis erectis tenuibus stramineis sursum hispidis 2-3 mm. longis.—Habitu specimen siccum foliis acriter dentatis nonnullis speciebus *Cirsii* simile.

Specimens examined: *C. Ledermann* 5440, growing 0.8-1.5 m. high at alt. of 1200-1400 m., Gendero, Pass Tchape, Kamerun, Oct. 5, 1909 (type in Herb. Berl.).

BIDENS PILOSA var. **MINOR umbrosa** f. nov.—Herba gracilis. Folia valde membranacea, 3-5-partita foliolis ovatis vel rhomboideo-ovatis, paucidentatis. Capitula minute radiata, pansa ad anthesin tantum circ. 6-7 mm. lata et 5-6 mm. alta. Flores ligulati circ. 4 vel 5, flavidi, circ. 4 mm. longi. Achaenia circ. 18-24, linearia, nigra, biaristata, 6-10 exteriora clavata atro-rubida corpore tantum circ. 4-5 mm. longa aristis demum saepe caducis, interiora nigra magis attenuata corpore 9-14 mm. longa.

Specimens examined: *Christian J. W. Schiede*, in shady places between Las Trojes and Hacienda de la Trinidad, State of Michoacan, Mexico, October (Herb. Berl., type and 2 cotype specimens).

BIDENS BIDENTOIDES var. **mariana** (Blake) comb. nov.; *B. mariana* Blake, *Rhodora* 31:88 and fig. 1. 1929.—In 1926, Dr. S. F. BLAKE submitted his specimens no. 9698 and 9703 to me for study. He had suspected them of representing a new species. Notwithstanding this, I felt compelled at that time to refer them to *Bidens bidentoides* (Nutt.) Britt. Later BLAKE published his *B. mariana*, based upon these and certain other plants. This has naturally led to a careful reinvestigation of the entire matter on my part. Especially has a survey of the closely related *B. hyperborea* and *B. connata*, together with their several varieties, been found helpful. In this way it became evident at once that *B. mariana* differs from *B. bidentoides* to about the same extent as, for example, *B. connata* var. *fallax* differs from *B. connata* var. *typica*. To recognize *B. mariana* as a species, therefore, would immediately (if consistency is to be sought) entail an elevation of numerous forms that FASSETT, FERNALD, FERNALD and ST. JOHN, and other writers (myself among them) have regarded as varieties. It has seemed wiser to adopt the alternative course and reduce *B. mariana* to varietal rank.

Bidens neumannii sp. nov.—Herba perennis, subsimplex, saltem 3 dm. alta, suberecta e radice lignescenti, breviter tomentoso-hispida, caule subtereti, internodiis medianis quam foliis brevioribus, inferioribus plurime tantum 5-12 mm. longis. Folia opposita, parce petiolata petiolis planis circ. 4 vel 5 mm. longis, omnino circ. 2.7-4 cm. longa et 1.1-1.7 cm. lata, indivisa, anguste oblongo-ovata, marginibus regulariter acriterque serrata dentibus indurato-apiculatis, apicaliter subacuminata. Capitula non numerosa (6 in unico speci-

mine observata), corymboso-paniculata pedicellis 1-7 cm. longis, radiata, pansa ad anthesin \pm 2-8 cm. lata et circ. 7-8 mm. alta. Involucri externe hispidi bractee exteriores 8-16, lineares, inferne saepe sensim angustatae, apice acriter indurato-mucronatae, demum circ. 6-8 mm. longae; interiores lanceolatae ventraliter nitidae et non nisi ad summam hispidae, paulo vel fere dimidio longiores. Flores ligulati circ. 10, saturate flavi, ligula elliptico-oblongi, apice bidenticulati, circ. 1.2-1.4 cm. longi. Paleae nitido-stramineae, lineari-oblongae, apice subabrupte mucronatae, nervo mediano perspicuae, achaenia superantes. Achaenia valde obcompressa, nigra, lineari-oblonga, unaquaque facierum circ. 8-striata, faciebus marginibusque adpresse erecto-setosa, non vere alata, corpore 4-5 mm. longa et circ. 1 mm. lata, apice erecte setoso biaristata aristis tenuibus sursum hispidis circ. 1-1.5 mm. longis.

Specimens examined: *Oscar Neumann* 135, alt. 2800 m., mountain meadows, Gardulla, Gallaland, Jan. 14, 1901 (type in Herb. Berl.).

***Bidens somaliensis* sp. nov.**—Herba perennis, erecta, supra erecte ramosa, verisimiliter circ. 9-12 dm. alta, caule glabrato tetragono. Folia opposita, non manifeste petiolata, 8-14 cm. longa et 1.2-3.3 cm. lata, lanceolata vel plus minusve spathulato-lanceolata, inferne sensim ad basim aegre connatam angustata, apice acuta sed non acuminata, acriter serrata plerumque 4-9 dentibus unico latere, subrigida, supra breviter hispida vel glabrata infra dense hispida. Capitula pedunculata pedunculis bracteatis inferne aegre superne valde hispidis circ. 1-1.6 cm. longis, radiata, pansa ad anthesin 4-6.5 cm. lata et 1.2-1.5 cm. alta. Involucri bractee plus minusve biseriales, exteriores circ. 10-12, lanceolatae vel ovato-oblongae, apice subacutae et induratae, marginibus ac saepe faciebus pilis albis articulis hispidae, circ. 0.9-1.2 cm. longae, in capitulo juniore disco subaequales; interiores oblongo-ovatae plus minusve hispidae, saepius paulo longiores. Flores radiati 8-14, flavi, ligula subanguste ovato-oblongi, apice minutissime denticulati. Paleae late lineari-oblongae, subrigidae, valde paucistriatae, supra saepe hispidae atque abrupte vel sensim angustatae, demum achaeniorum corpora paulo superantes. Achaenia cuneato-oblancoolata, plana, brunneo-nigra, faciebus sparsim marginibus atque apice dense erecto-setosa, unaquaque facierum nunc circ. 8-nunc circ. 16-sulcata, corpore 1-1.4 cm. longa

et 3-4.5 mm. lata, biaristata aristis acribus brunneo-stramineis, infra medium subsparsim antrorsum setosis supra medium plerumque nudis.

Specimens examined: *Dr. Domenico Riva* 85 (field no. 1306; *Expedition of Eugenio Ruspoli*), in grassy fields and on plains, from Biddum to Volghe, Somalia, Sept. 15, 1893 (2 type sheets in Herb. Florence).

Bidens taitensis sp. nov.—Herba erecta, verisimiliter perennis, glabra vel sparsissime hispida, forsitan 1 m. alta. Folia opposita, principalia petiolata petiolis marginatis ± 1.5 cm. longis, petiolo adjecto ± 9 cm. longa, circumambitu late deltoideo-ovata, bipinnatisecta, foliolis (lateralibus 2 jugis) membranaceis, subgrossé dentatis, decurrentibus, saepius oblongo-lanceolatis vel ovato-lanceolatis, minute nigro-punctatis, segmentis apicaliter subacutis. Capitula tenuiter pedunculata pedunculis glabratissimis vel ad summam hispidis, ± 1 dm. longis, radiata, pansa ad anthesin probabiliter 3-5 cm. lata et 8-10 mm. alta. Involucri bractee exteriores circ. 8 vel 9, late oblongae, apice obtusae cartilagineo-indurataeque, marginibus eciliatae, tergo nonnullis lineis nervatae, circ. 8-9 mm. longae et 2-4 mm. latae; interiores late oblongo-lanceolatae dorsaliter subsparsim hispidae, apicaliter dense pubescentes, saepe paulo breviores. Flores ligulati (tantum unicus visus) flavi, ligula lineari-oblongi, nervis pilosi, apice subintegri, ± 1.5 cm. longi. Achaenia nunc late cuneato-lineariter nunc oblongo-oblancheolata, plana, atra, unaquaque facierum circ. 8-striata, non vere alata, marginibus dense faciebus non nisi supra erecto-setosa, corpore 6-8 mm. longa et 1.5-2.2 mm. lata, apice erecte hispida et concavo vel raro plano exaristata vel brevissime biaristata aristis usque ad 0.5 mm. longis nunc calvis nunc sursum 1-3- hamosis.

Specimens examined: *J. M. Hildebrandt* 2432a, alt. 2000-3000 ft., Taita (Teita) Mts., British East Africa, February, 1877 (type in Herb. Berl.).

Hildebrandt's no. 2432 was collected in quantity and proved to be new (*Bidens hildebrandtii*). Specimens were distributed to Kew, London, Berlin, Vienna, and elsewhere. His 2432a, however, although collected at the same time and place, seems to have been rare. Apparently only a single specimen was found, and that none too ample. The habit seems nearest that of *Bidens fischeri* (O. Hoffm.) Sherff, of

German East Africa. From that species it differs, however, in being more glabrous, in having the exterior involucre bracts also the achenes much wider, etc.

BIDENS BIPINNATA *bitermatoides* var. nov.—Folia pinnata foliolis lateralibus 3-4-jugis, imis tripartitis segmentis lanceolatis, caeteris simplicibus lanceolatisque. Capitula discoidea vel subligulata. Involucrum bracteae exteriores superne sensim vel interdum fere subabrupte dilatatae. Achaenia 2-aristata.

Specimens examined: E. O. WOOTON, in cultivated land, Las Cruces, New Mexico, October, 1895 (type in Herb. N.Y. Bot. Gard.).

In its slightly dilated outer involucre bracts this variety makes an approach toward *Bidens pilosa* L. The general aspect of the plant is at once that of the South American *B. subalternans* DC. and of the Old World *B. bitermata* (Lour.) Merr. & Sherff, from both of which it differs sharply, however, in its achenes.

Coreopsis negriana sp. nov.—Herba annua, crecta, ± 5 dm. alta, caule gracili, glabro vel ad summam subhispidulo, viridi-stramineo. Folia opposita petiolata petiolis usque ad 12 mm. longis, saepe tenuissimis, petiolo adjecto ± 5 cm. longa, 1-2-pinnatisecta, segmentis principalibus lineari-oblongis, membranaceis, supra infraque glabris, marginibus ciliatis, acriter serratis dentibus interdum in setas elongatas desinentibus. Capitula graciliter pedunculata pedunculis moderate hispidis usque ad 14 cm. longis, radiata, pansa ad anthesin 2-2.5 cm. lata et circ. 6-7 mm. alta. Involucrum hispidi bracteae subaequales exteriores circ. 8, lineari-spathulatae, acriter indurato-apiculatae, 3-4 mm. longae; interiores oblongo-ovatae, apicaliter saepe acutae. Flores ligulati circ. 8, flavi, ligula lineari-elliptici, apice obtuso obscure denticulati, circ. 1 cm. longi et circ. 2-3 mm. lati. Paleae nitido-hyalinae, lineari-oblongae vel lineari-lanceolatae, demum conduplicatae ac achaenium amplectentes, maturae circ. 6 mm. longae. Disci florum stigmata subito capilliformi-caudata. Achaenia lineari-oblonga, valde obcompressa, glabra, corpore nigro estriato vel obscurissime multistriato tantum 3-4 mm. longa et circ. 1 mm. lata, marginibus anguste alata alis brunneis glabrisque, apice exaristata ac glabra.

Specimens examined: Dr. Giovanni Negri 915 bis, alt. 1500 m., Arussi Galla, June 30, 1909 (Herb. Flor., type).

A species close to *C. pachyloma* O. & H., from which it differs, however, in its smaller capitula, also its smaller achenes which are obcompressed, winged, and glabrous. The general habit is deceptively like that of *Bidens setigera* (*Coreopsis setigera* Schz. Bip.), some of the foliar teeth even having the setiform extensions so notable in that species. But *B. setigera*'s achenes are distinctly striate, upwardly setose upon the faces and edges, entirely exalate, and at the apex conspicuously slender-aristate.

CHICAGO NORMAL COLLEGE

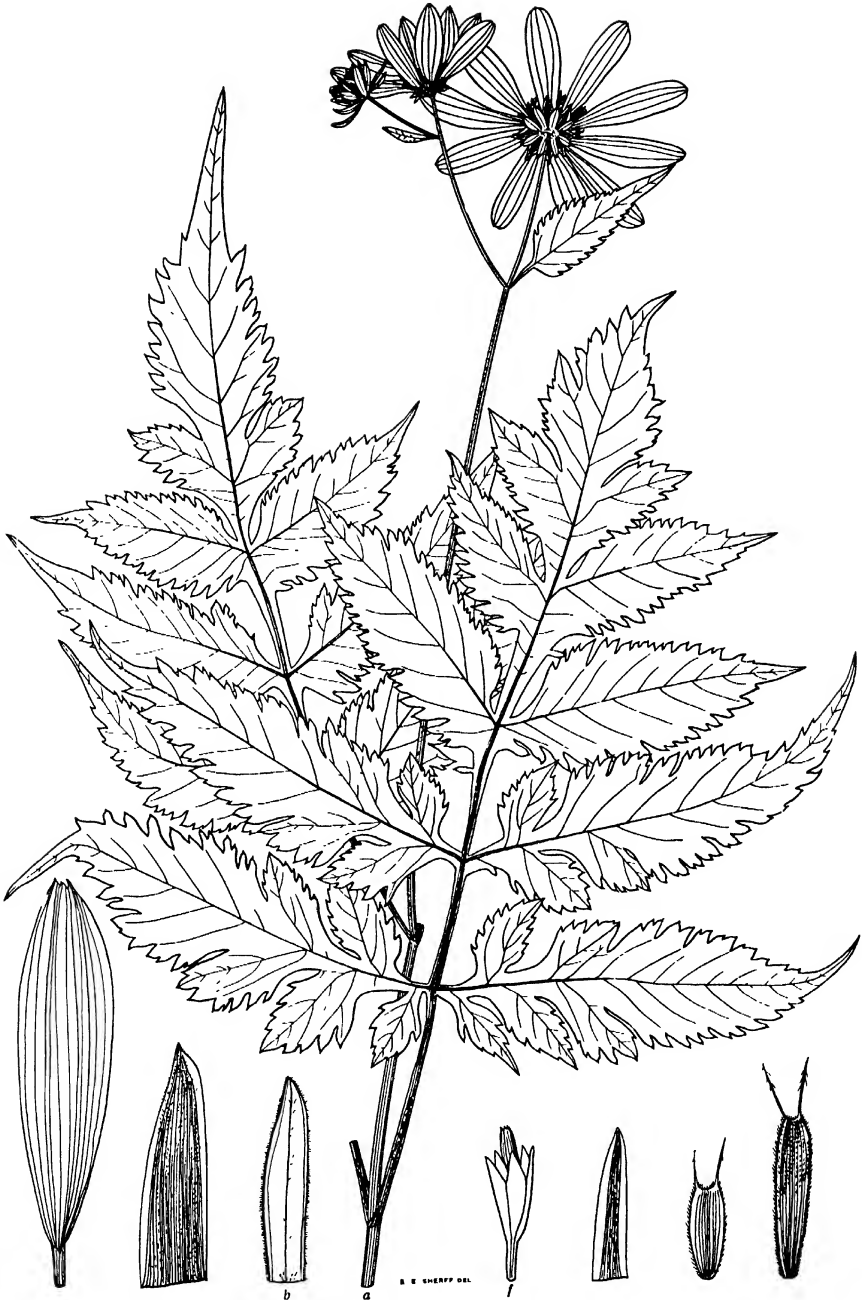
EXPLANATION OF PLATES IV, V

PLATE IV

Bidens magnifolia: *a*, flowering branch, $\times 0.56$; *b*, exterior involucre bract, $\times 3.36$; *c*, interior involucre bract, $\times 3.36$; *d*, ray floret, $\times 2.8$; *e*, palea, $\times 3.36$; *f*, disc floret, $\times 3.36$; *g*, exterior and *h*, interior achenes, $\times 4.48$; *g* and *h*, from *Albers* 204 in Herb. Berl.; rest from *Holst* 2252, type in Herb. Berl.

PLATE V

Bidens cirsioides: *a*, *b*, lower and upper portions of one flowering branch, $\times 0.61$; *c*, exterior involucre bract, $\times 3.66$; *d*, interior involucre bract, $\times 3.66$; *e*, ray floret, $\times 2.44$; *f*, palea, $\times 3.66$; *g*, disc floret, $\times 4.88$; *h*, achene, $\times 4.88$; all from *Ledermann* 5440, type in Herb. Berl.



B. E. SHERFF DEL.

SHERFF on COMPOSITAE



SOME EFFECTS OF LOW TEMPERATURES ON SEEDS¹

W. F. BUSSE AND C. R. BURNHAM

(WITH THREE FIGURES)

Introduction

It has long been known that air-dry seeds retain their viability after freezing to -190° C. in liquid air (1), but little work has been done to determine whether this treatment has any effect on the later growth of the plant. In experiments carried on in this laboratory, it has been found that many plants, such as maize, wheat, barley, lettuce, onions, and radishes, grow normally after the seeds have been frozen in liquid air. Germination of clover and alfalfa is markedly improved by this treatment (2). With cotton and flax, however, there are some interesting differences between the plants from treated and untreated seeds. This paper is a report of some abnormalities found with these seeds.

I. Cotton

When cotton seeds germinate normally, the hypocotyl grows out of the tip and the cotyledons are gradually pulled out of the seed-coat. Occasionally the cotyledons have difficulty in getting free, but it is nearly always possible in these cases to remove the seed-coat by hand without injuring the cotyledon. If the seeds are cooled with liquid air, either by placing in a container which is then immersed in the liquid air or by placing directly into the air, and are then germinated, the result is different. The percentage of seeds germinating is not changed nor is growth of the hypocotyl greatly affected, but in every case the cotyledons have great difficulty in getting out of the seed-coat. Even when the plant finally succeeds in shedding its coat, the cotyledonary leaves are filled with cracks which look as though the cotyledons had been made brittle while

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FIG. 1.—Effect of freezing cotton seeds in liquid air: left pot, control; right pot, treated

the seed was at the liquid air temperature. Usually the seed-coat is pushed only part way off, and any effort to remove it by hand results in tearing off part of the already cracked and broken cotyledonary leaves. Fig. 1 shows two lots of cotton plants grown under identical conditions, except that the plants in the second pot were from seeds which had been frozen in liquid air, while the plants in the first pot were controls. It will be noted that the cotyledons of every plant in



FIG. 2.—Leaves from normal cotton seedlings (left) and from seedlings produced by seeds frozen in liquid air (right).

the second pot are injured, while the controls are normal. Fig. 2 gives a better view of typical leaves from the controls and from plants grown from treated seeds.

The secondary leaves which came out were normal, even though the cotyledonary leaves were badly injured. This seems to indicate that the injury is a purely mechanical one, acting only on the leaves which are already developed. The plants were not grown to a height of more than 25 cm. as the experiments had to be stopped at this stage.

II. Flax

When pure strains of flax seeds were treated with liquid air, it was found that there was little injury to the cotyledonary leaves, al-

though a few of them split along the middle vein. Instead of growing with a single stem in the seedling stage, however, as the control plants did, some of the seedlings from treated seeds grew differently. Nearly all started with a single stem, but this often became split into two separate stems anywhere from two to fifteen nodes (about



FIG. 3.—Flax seedlings grown from seeds treated with liquid air

1-7 cm.) above the crown of the plant. In even more cases a fasciated and split hypocotyl developed from the seeds treated with liquid air.

Fig. 3 shows the type of plants which developed. The three plants at the left are normal, each having a single stem and a single hypocotyl. The next two plants have twinned stems and single hypocotyls, while the two following have both the stem and the hypocotyl doubled. The last two plants have single stems but double hypocotyls. In one experiment only one plant out of about 20,000 grown from pure lines of untreated seeds showed any fasciation or doubling,

while in some cases freezing in liquid air resulted in more than 70 per cent with these abnormalities. In an attempt to find the factors causing this effect, the following experiments were performed.

VARIETAL DIFFERENCES AND EFFECT OF RATE OF WARMING
ON AMOUNT OF TWINNING

Ten different strains of flax were used, including Argentine, Indian, Ottawa White-flowered, Abyssinian Yellow-seeded, Crete, and Saginaw selections. These are all seed flaxes except the last, which is a fiber type. To be certain that the strains were pure, lines were used in which natural crossing had been guarded against by bagging individual plants for a number of generations. Two lots of 50 seeds each were treated, and one lot of 100 seeds from each line was used as a control. The treated seeds and controls were planted in alternate rows on a greenhouse bench.

It was noted in preliminary experiments that when the seeds were taken out of the liquid air and immediately placed on a piece of metal to warm up quickly, the mechanical stresses in the seed were great enough to cause it to burst and shatter the outer seed-coat, sending the rest of the seed flying several feet but apparently not injuring it. It was thought that this violent reaction might be associated with twinning of the stems, so two different treatments were given the seeds. Treatment *A* consisted of placing the seeds in liquid air in a Dewar flask and allowing them to remain there until the liquid air had evaporated and the flask and the seeds had warmed up to room temperature, thus giving a very slow warming of the seeds. In treatment *B* the seeds were cooled by placing in liquid air for two or three minutes and then were warmed up as rapidly as possible by taking them out of the liquid air and immediately placing them on a metal plate.

The results of these experiments are shown in table I, which gives the germination, the percentage of plants having double stems, the percentage having a twinned hypocotyl, the percentage having both, and the total percentage having either an abnormal stem or hypocotyl or both. The last three columns give the percentages of plants having extra leaves, such as three or more at a node, or two on one side of the stem at a node, and also include abnormal leaves such as

TABLE I
EFFECT OF FREEZING DIFFERENT STRAINS OF FLAX IN LIQUID AIR

VARIETY	PERCENTAGE GERMINATION				TOTAL PLANTS		PERCENTAGE												
	A		B		A	B	DOUBLE STEM AND SINGLE HYPOCOTYL		SINGLE STEM AND DOUBLE HYPOCOTYL		DOUBLE STEM AND DOUBLE HYPOCOTYL		TOTAL ABNORMALITIES		IRREGULAR LEAVES				
	Con- trol	A	B	A			B	Con- trol	A	B	Con- trol	A	B	Con- trol	A	B			
6. Crete...	79	68	66	33	34	33	0	3	6	0	3	0	0	0	9	12	0	0	6
9. Abyssinian...	56	16	28	14	8	14	0	0	0	0	0	0	0	0	0	0	0	0	0
11. Ottawa...	93	90	76	45	38	38	0	0	4	0	0	0	0	0	0	4	8	10	10
15. Indian...	86	90	94	45	47	47	0	2	0	0	0	0	0	0	2	2	10	4	16
16. Argentine...	89	86	88	43	44	44	0	5	2	0	0	0	0	0	5	2	15	30	22
20. North Dakota...	99	98	94	49	47	47	0	2	0	0	0	0	0	0	2	0	1	4	10
21. Argentine...	98	64	56	32	28	28	0	13	4	0	16	18	0	0	0	38	22
22. Argentine...	97	72	62	36	31	31	0	20	9	0	6	9	0	0	0	32	18
22. Argentine...	93	51	62	51	62	62	0	4	8	1(?)	40	8	0	26	37	5	20	46	...
8A. Common seed...	86	86	74	43	37	37	0	0	2	0	0	2	0	0	0	4
D5. Saginaw...	86	62	70	31	35	35	0	3	3	0	19	3	0	0	22	6
8A. Common seed*	67	54	88	27	44	44	0	0	4	0	0	2	0	0	0	6
11. Ottawa*	68	58	54	29	27	27	0	0	4	0	0	0	0	0	0	4
22. Argentine*	56	30	34	15	17	17	0	20	12	0	0	12	0	0	26	24

* Grown in botany department greenhouse; others grown in genetics department greenhouse.

semidouble ones (having two main veins and tips but failing to separate farther back of the tips). The table shows that germination of some of the lots is reduced by the liquid air treatments, but there is no absolute correlation between lowered germination and twinning. There are marked varietal differences in the amount of twinning, suggesting a genetic or heritable difference between strains; but the type of treatment has surprisingly little effect.

The last three samples shown in table I were given the same treatments as the others, but were grown in a different greenhouse to make sure that it was not some peculiar local condition of the soil or atmosphere that was causing the results. While the amount of twinning found here was somewhat less than in the other tests with the same lots of seeds, the results are of the same order in both cases. Only one plant from the 1400 untreated seeds planted as controls in this series showed any suggestion of forming a fasciated or doubled stem or hypocotyl, while in some cases 30-70 per cent of those treated showed double stems or hypocotyls. This would indicate that the phenomenon is due to treatment of the seeds with liquid air.

EFFECT OF TIME BETWEEN TREATMENT AND PLANTING ON AMOUNT OF ABNORMALITIES

Since it is known (3) that even comparatively simple protein systems, such as gelatin solutions, sometimes require days to reach equilibrium after thermal changes, it was thought that the time between thawing of the seed and planting might influence the formation of twinned stems and hypocotyls. To test this, three 50-seed samples of lot 22 were given treatment *B* and one was planted 4 hours later, one 3 days later, and the other 2 weeks later, planting controls with each lot of treated seeds. The results are given in table II.

In the lot of seeds planted 2 weeks after treatment, germination was markedly slower than in controls planted at the same time. Table II shows that the germination percentage was low in all treated lots, but the formation of abnormalities is not greatly affected by storing the seeds two weeks after freezing in liquid air.

GROWTH OF PLANTS FOLLOWING TREATMENT

On the average, plants from treated seeds were not so tall as those from controls, and the abnormal plants were shorter than the normal

ones from treated seeds, as shown in table III. This table gives the heights of plants in the series reported in table II. With the exception of the seeds planted 2 weeks after treating, the amount of variability is much greater in the abnormal plants from the treated

TABLE II

EFFECT OF TIME BETWEEN FREEZING SEEDS AND PLANTING, LOT 22; TREATMENT B

TIME BETWEEN FREEZING AND PLANTING	PERCENTAGE GERMINATION		TOTAL PLANTS TREAT- ED	PERCENTAGE					
				DOUBLE STEM		DOUBLE HYPO- COTYL		TOTAL ABNOR- MALS	
	Control	Treated		Control	Treated	Control	Treated	Control	Treated
4 hours	93	62	62	0	16	1	45	1	53
3 days	93	67	67	0	23	0	44	0	52
2 weeks	89	57	57	0	30	1	39	1	54

TABLE III

COMPARATIVE HEIGHTS IN CENTIMETERS OF NORMAL AND ABNORMAL PLANTS

TIME BETWEEN FREEZING AND PLANTING	MEASUREMENT	CONTROL	TREATED SEEDS			
			Normal	Single stem, double hypo- cotyl	Double stem, single hypo- cotyl	Double stem, double hypo- cotyl
4 hours	Mean height	17.5 ± 0.25	16.6 ± 0.62	12.9 ± 0.59	13.1 ± 1.23	9.6 ± 0.49
	Standard deviation	3.26	3.94	3.95	4.54	2.87
	Coefficient of variability	18.6 ± 1.06	23.7 ± 2.66	30.6	34.7	29.9
3 days	Mean height	Same as above	14.8 ± 0.52	14.0 ± 0.48	10.7 ± 1.17
	Standard deviation		4.16	3.51	3.93
	Coefficient of variability		28.0	25.1	36.7
2 weeks	Mean height	15.3 ± 0.22	15.3 ± 0.39	12.4 ± 0.48	11.8 ± 0.72	10.9 ± 0.42
	Standard deviation	2.62	3.22	3.08	2.42	1.97
	Coefficient of variability	19.4 ± 1.20	21.0	24.8 ± 2.96	20.5	18.1

lots than in the control. The number of plants having the different types of abnormalities is too small to make it possible to draw any conclusions as to the type of abnormality that is usually associated with the greatest dwarfing.

EFFECT OF MOISTURE CONTENT OF SEEDS

It was thought that the amount of moisture in the seeds when they were frozen might have an effect on the formation of abnormali-

ties, so seeds of different moisture content were treated with liquid air. Two 100-seed samples of each variety were dried 10 hours in a drying room at 51° C., and the moisture content of the other seeds is recorded as the amount above that present in this dry state.

To increase the moisture content, the seeds were placed in an atmosphere of high humidity until the stated amounts of moisture had been taken up and they were then kept in stoppered 2-inch

TABLE IV
EFFECT OF MOISTURE CONTENT ON FORMATION OF ABNORMALITIES
FROM FLAX SEEDS TREATED WITH LIQUID AIR

VARIETY	PERCENTAGE						
	MOISTURE	GERMINATION		DOUBLE OR FASCIATED HYPOCOTYL		DOUBLE GROWING POINT	
		Treated	Control	Treated	Control	Treated	Control
22	0.....	77.3	89 1	68 3	0	15 9	0
	5.. . .	82 6	90 0	17 6	1 0	6 8	0
	6 .. .	85.5	. . .	39 3	. . .	3 4	. . .
	7.	84 5	. . .	18 3	. . .	7 3	. . .
	10 .. .	80 1	91.3	1 1	0	2 2	0
	15.....	97 3	. . .	0	0	. . .
11	5.....	84 5	88.2	1 0	0	0	0
	7.	93 6	0	. . .	0	. . .
	10....	92 7	0	0	. . .
	15 .. .	65.5	0	0	. . .

test-tubes. The treatment was made by immersing the tubes in liquid air. Two lots of seeds were used, no. 22, which usually gave a high percentage of abnormalities, and no. 11, which gave almost no abnormalities when the seeds were frozen. The results are shown in table IV. These results show that the percentage of abnormal plants of strain no. 22 decreases with increasing moisture content of the seeds up to 10 or 15 per cent moisture. The germination percentages show that liquid air treatment has not killed the seeds of this lot having moisture contents up to 15 per cent. Strain no. 11 showed decreased germination when seeds containing 15 per cent moisture were frozen in liquid air, but there were practically no abnormalities when the seeds of either strain having this moisture content were frozen.

EFFECT OF FREEZING IN CO₂ SNOW

To determine whether less intense freezing could cause these same abnormalities, samples of strain 22 having various amounts of moisture were placed in 2-inch test-tubes and immersed in a CO₂-ether mixture (-80° C.) for 10 minutes. They were then removed and planted the following day. The results of this treatment are shown in table V. A comparison with table IV shows that CO₂ snow

TABLE V

EFFECT OF FREEZING IN CO₂ SNOW ON SEEDS OF VARIOUS MOISTURE CONTENT

VARIETY	PERCENTAGE						
	MOISTURE	GERMINATION		DOUBLE OR FASCIATED HYPOCOTYL		DOUBLE GROWING POINT	
		Treated	Control	Treated	Control	Treated	Control
22	0 . . .	73 6	89.1	68 3	0	15 9	0
	5 . . .	100 0	90.0	47 0	0	17 6	0
	7 . . .	90 9	.	38.8	10.0
	10 .	97 3	91 8	1 3	0	0 0	0

is even more effective than liquid air in causing abnormalities, and like liquid air it is more effective with the drier seeds.

BREEDING BEHAVIOR OF PLANTS FROM TREATED SEEDS

Thirty normal plants from treated lots giving abnormalities were grown to maturity in the field; together with five plants having double stems and single hypocotyls and three having double stems and double hypocotyls.

Fifty to one hundred seeds from each of these plants were planted in the greenhouse and observed for the presence of abnormalities. In a total of over 3000 plants, only two with forked stems were noted, and 17 plants with fasciated and two with double hypocotyls were observed. In about 2000 seedlings from control or untreated seeds, five with fasciated hypocotyls were found and none with double stems or hypocotyls. Thus there is no significant difference between the treated and untreated lots. The abnormalities produced by the low temperature treatment are not inherited, but seem to be due to temporary changes in the developmental processes.

Discussion

The results of these experiments raise some interesting questions as to the mechanism by which freezing a dormant seed will, at some later date, cause the growing point of the seedling to develop in two directions, producing doubling of the stem. While it is not possible to give a final answer to these questions, some factors which might be affecting the process may be discussed.

The fact that some pure lines of seeds give a large percentage of abnormal plants when frozen in liquid air or solid CO₂-ether mixture, while other pure lines give few or none when subjected to the same treatments, seems to show that the phenomenon has some genetic basis. These lines have been inbred for so long that the seeds of any one strain should be genetically alike. When they are frozen, however, some seeds of a strain show no apparent effect while others produce abnormal plants. This may be due to variations in external and internal environment and therefore non-genetic. The seed bolls of flax are produced over a long period, and conditions during seed development, such as temperature, moisture, available nutrients, certainly are not the same for all seeds on a plant. In this connection it may be worth noting that plants of nearly all the pure lines show variations in the number of nodes, with two leaves that are produced before the leaves start to grow with just one at a node. Also some of the plants produce extra or double leaves. Unfortunately data were not taken on the double and extra leaves in all cases, but table I gives some evidence that when freezing the seeds causes much twinning of the stem and hypocotyl it also increases the percentage of plants having abnormal leaves. If this is generally true, then exposing the seeds to low temperatures may liberate an inherent tendency of the embryonic cells to separate and form more parts than are usually produced.

Whether this view is true or not, the mechanism by which the change is brought about is probably a physicochemical one. The effect might be due to the simple mechanical stresses and strains set up in the seed owing to different coefficients of expansion and different rates of cooling of the different parts. When these stresses are great enough to burst the seed-coat with a force sufficient to send the seed flying for a distance of several feet, it is evident that the struc-

ture within must be subjected to severe strain, but it is rather difficult to see just how it could affect the cells in the growing point to make them separate and later produce two stems or two hypocotyls.

If the different coefficients of thermal expansion of the different parts of the seed cause what might be called an "inner crack" at the tip of the growing point, this should cause the point to develop into two separate parts. If this is so, it might be possible to produce the same results by very careful micro-probing of the seed. The stresses might also produce a direct mechanical injury to the cells at the tip of the growing point, and when the other cells grow around these, two tips would form.

Another possibility is that freezing alters the protoplasm of the individual cells in such a way that the cells of the growing point fail to respond normally, thus allowing the production of several growing points and hypocotyls as well as abnormal leaves. The fact that most dry seeds are uninjured by freezing makes it easy to lose sight of the fact that freezing might cause injury to the protoplasm in some cases, even when there is not enough water to separate out as ice crystals. MORAN (4) showed that freezing and thawing a gelatin gel caused permanent changes in its volume and in its ability to absorb water. HARDY (3) showed that while normally gelatin gels are singly refractive, part of the gel becomes doubly refractive on freezing and remains so after thawing.

If such effects are observed in this simple system, one must consider the possibility of much more diverse and striking effects in a complex system like protoplasm. It may be that some such change in the protoplasm of the seeds is produced by the freezing, and this causes the formation of twin stems and hypocotyls as well as double and extra leaves. It would be surprising to find flax the only plant thus affected by intense freezing of the seeds, so it should be worth while to see whether other plants would not also produce the same type of effect when the seeds are treated with liquid air. If other such cases were found they might throw more light on the cause of the effect in flax.

Summary

1. Freezing cotton seeds in liquid air injures the cotyledons so that they become cracked and split and are not able to emerge completely

from the seed-coat. The secondary or true leaves do not appear to be injured by treating the seeds with liquid air.

2. When flax seeds of certain strains are treated with liquid air, a large proportion of the plants form double stems and hypocotyls; none of the plants from untreated seeds do this. Other strains rarely if ever produce plants with double stems or hypocotyls when the seeds are cooled in liquid air.

3. The time between freezing the seeds and planting them has little effect on the formation of abnormalities.

4. Increasing the moisture content of the seeds before freezing them reduces the number of plants having abnormal stems and hypocotyls.

5. Freezing the seeds in a CO₂ snow-ether mixture (−80° C.) is somewhat more effective in producing abnormalities than freezing them in liquid air (−190° C.). As with the liquid air, no abnormalities are produced when seeds having about 10–15 per cent moisture are frozen in CO₂ snow.

6. Breeding tests showed that the abnormalities produced are not inherited.

7. The mechanism by which the abnormalities are produced cannot be deduced from the data available, but it seems probable that freezing causes some physicochemical change in the protoplasm of the embryo cells which causes them to react differently to the normal stimuli; or it changes some of the restraining and formative forces, which cause the plant to develop in a more or less fixed way.

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HYDROLYSIS IN THE LIVING PLANT BY POLARIZED LIGHT¹

ELIZABETH SIDNEY SEMMENS

(WITH NINETEEN FIGURES)

Introduction

During the past five years, a series of simple experiments has been carried out, showing that polarized light has the power of hydrolyzing starch and other reserve products within the leaf of the living plant.

These experiments have been performed in England, Canada, and South Africa, under widely differing conditions of temperature and light intensity, and have given definitely positive and consistent results. Simple as these experiments appear, they nevertheless indicate a fact of great importance in plant metabolism, for all plants, particularly if the sky is clear, are exposed to a constant succession of polarized and non-polarized light.

I. Hydrolysis by polarized light

It is well known that the morning and evening sky is strongly polarized, the maximum polarization lying along a great circle having the sun for its pole, as indicated by testing with a Nicol's prism. It has been found that this light has a decidedly hydrolyzing effect upon starch, glucosides, and other complex organic reserve substances within the leaf, at temperatures considerably below those ordinarily associated with enzyme action.

If a healthy young leaf is exposed to light, polarized either naturally, as in skylight under certain conditions, or artificially by reflection or by transmission through a Nicol's prism, the starch in the mesophyll is found to break down in the area thus exposed; and on staining with iodine a light patch is seen, corresponding in position and shape with this area. There is no doubt that this is due to the orientation of the molecular surfaces. Since plants and animals consist

¹ Paper read at the British Association Meeting in South Africa; July, 1929.

almost entirely of definitely oriented surfaces: starch grains, blood corpuscles, cell walls, membranes, in this selective action of polarized radiation, whether of heat or light, we are dealing with some correlation between the vibration direction of the incident radiation and the orientation of the electron orbit of the molecules at the surface.

The writer's attention has recently been drawn to an investigation² which attempted the impossible task of obtaining differential results with polarized light on boiled starch solution, contained in a small vessel, when negative results were obtained. But why should there be any selective effect on the uncontrolled molecules in a test-tube?

The writer, when working in Professor E. C. BALY's laboratory at Liverpool in 1923, also made a series of very careful experiments upon boiled starch. The selective results were extremely small, and it was not felt that they were suitable for publication at the time, but these negative results led to important conclusions and paved the way for further experiments, of which a full account will be given later.

BUNKER and ANDERSON also experimented with starch grains, but as they refer only to unpublished data it is difficult to estimate the value of their results. It may suffice to suggest that, as all their experiments were done at temperatures over 40° C., in the presence of diastase there could scarcely be room for any differential effect.

The writer has always worked at temperatures much below those favorable to enzyme action, and it was stated (British Association Report, 1923) that at 20°–25° C. the selective effect disappeared.

Experimentation

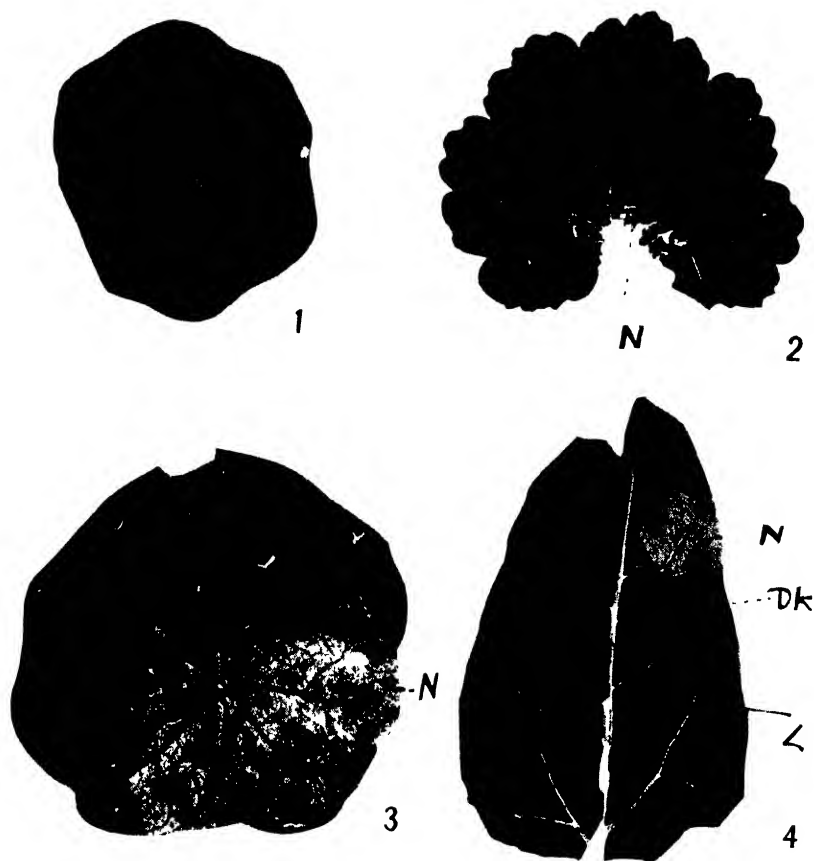
To demonstrate hydrolysis in the leaf, experiments were arranged as follows.

A young and healthy dicotyledonary leaf (preferably of thin texture and without hairs, which have a scattering and depolarizing effect) was exposed to blue skylight, behind a Nicol's prism having a wide rim. The instrument rested lightly against the leaf, which was kept from shifting either by a support to the stem or by a soft cotton pad just touching the back of the leaf. Care was taken to

² BUNKER, J., and ANDERSON E., Jour. Biol. Chem. May. 476–487. 1928.

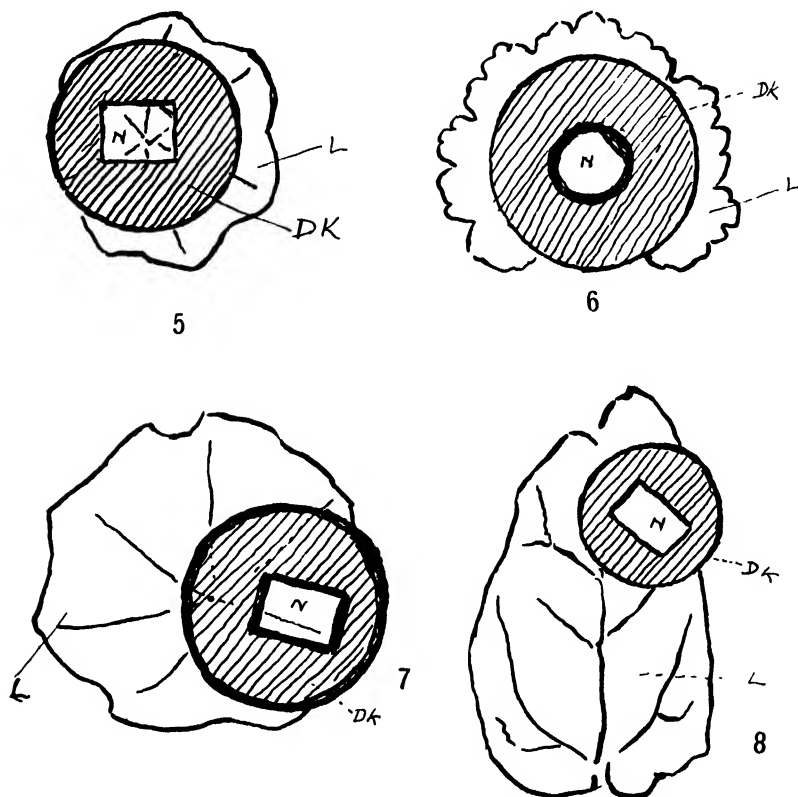
insure that the air was able to circulate freely over the leaf, providing free interchange of gases and equality of air temperature.

The experimental area and two controls, one in comparative darkness (*DK*) and one in full daylight (*L*), were on the same leaf. A



FIGS. 1-4.—Fig. 1, *Tropaeolum* leaf exposed on living plant in E by NE window of cool upper laboratory in Bedford College, London, June 4, 12:30 to 6:30 P.M.; starch remained unchanged in dark but hydrolyzed under polarized light; fig. 2, smooth geranium leaf exposed in cool greenhouse, March 13, London, for 3 hours; starch seen under Nicol's prism; fig. 3, *Tropaeolum*, June 20, College garden, 6 hours; part exposed to daylight is darkened, owing to 6 hours more photosynthesis than *DK*, but part in polarized light is much lighter; fig. 4, spinach leaf, South Africa, College grounds, December 17, 12:45-7:00 P.M., bright hot weather; hydrolysis in *N* and photosynthesis over *L* are very strong (in both figs. 3 and 4 there is probably some hydrolysis in *DK*, owing to stimulation of leaf enzyme by higher day temperature).

careful drawing of the exact position of the Nicol was made and kept with details for reference. After 4-6 hours' exposure, the leaf was picked and one or two pin-holes made to confirm the position of the Nicol. The leaf was then boiled in 95 per cent alcohol, or



FIGS. 5-8.—Illustrating shape and position of polarizing Nicol in each case: *N*, area of polarized light behind Nicol aperture; *DK*, area in darkness, behind opaque rim of cork, etc. *L*, area exposed to daylight.

steeped all night and then dipped in iodine in potassium iodide and mounted in glycerin.

As shown in the photographs (figs. 1-4), a light area, exactly defining that exposed to light through the Nicol, was seen. The outer part was very dark, owing to photosynthesis, and the part under the rim in comparative darkness remained unchanged at ordinary

low temperature; but if the weather was warm there was slight diminution of starch, due no doubt to the slow action of the enzyme in the leaf. The rate of starch hydrolysis, therefore, even at comparatively high temperatures, is shown to be many times greater under polarized light than in darkness.

To show that no other factor, such as pressure, temperature, etc., was producing this effect, a further control experiment was made. A leaf was exposed in a similar manner, but the crystal of the Nicol was replaced by a piece of glass. As was expected, instead of the lighter area due to hydrolysis, a dark patch was seen in the center, indicating photosynthesis by the non-polarized light.

HYDROLYSIS OF ANTHOCYANIN.—Observations on young seedlings exposed to polarized light showed a diminution of red coloring matter, and the preceding experiments were therefore repeated with leaves of the beet. A deep crimson leaf of *Beta vulgaris*, of uniform tint, was chosen and over it was placed a Nicol's prism, the back of the leaf resting gently against another leaf and being held lightly in position. After 6 hours' exposure the leaf was picked, plunged into dilute sulphuric acid, and then laid in amyl alcohol for several hours.

Result

At the point of exposure drops of red liquid (anthocyanidin) could be seen dissolving into the amyl alcohol, and after some hours a light patch was seen, taking the shape of the exposed area, indicating that the anthocyanin on the exposed upper surface had hydrolyzed to anthocyanidin and had been dissolved by the amyl alcohol.

In performing these experiments, considerable patience and care are necessary. Too long exposure may result in degradation of the protoplasm and a dark brown spot is seen in the middle of the light area (*N*). The whole tissue may collapse, or the plant be killed by too long exposure. Too short exposure or too weak light may result in the starch changing only to erythro-dextrin, and giving a brownish patch; but if the leaf is washed in hot water and again flooded with iodine solution, this is eliminated and the light patch appears. Great care must be taken in adjusting the Nicol's prism, so as to prevent the oblique non-polarized beam from passing through to the leaf.

The effect can be obtained only with young and healthy leaves.

The leaves chosen for exposure were *Tropaeolum*, *Fuchsia*, *Geranium* (smooth leaf), sow-thistle, spinach, lucerne, and others, all of which gave striking differential results, showing starch hydrolysis under the polarized radiation.

Exposure of a hairy leaf of *Abutilom* gave only a faint outline of the Nicol aperture, showing that the hairs had partly depolarized the light.

Nearly one hundred of these experiments have been made, and a few photographs of results are shown (figs. 1-4).



FIG. 9.—Leaves from bed of *Tropaeolum*: *P*, picked at sunset; *DK*, kept in dark and picked at 9:00 A.M. next morning (19 hours).

II. Action of blue skylight

CANADA.—The first experiments were made during a brief stay in Canada, in the month of September.

A bed of *Tropaeolum* was chosen which was lying to the east of a tall building, and thus shielded from the rays of the setting sun but exposed to full sunlight until about 2:00 P.M.

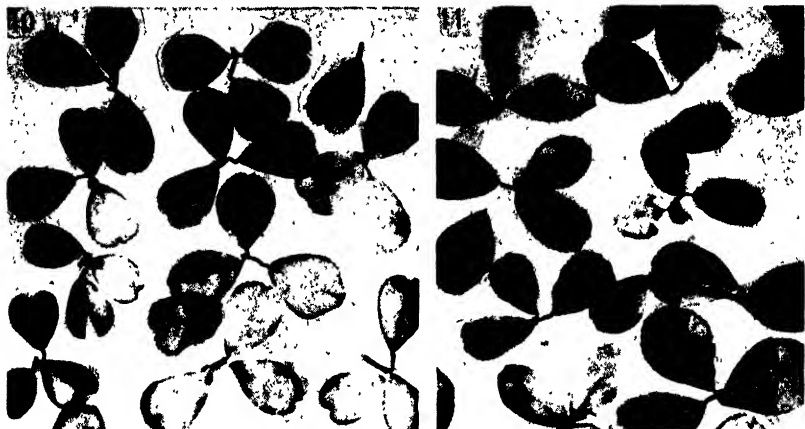
On a cloudless day a small spray of young leaves was covered at this hour and left till 9:00 A.M. the next morning, when it was picked and plunged into 95 per cent alcohol. A young adjacent leaf, having the same aspect, was noted at the same time but picked at 6:00 P.M. on the first evening, having thus been exposed for 4 hours to the polarized afternoon skylight.

On staining with iodine, this second leaf (fig. 9 *P*) was found to be almost denuded of starch, while the leaves (fig. 9 *DK*) which had

been in darkness for 19 hours had full starch content. The experiments were carried out in comparatively cold weather, but after a day of torrential rain, followed by a slight frost, no further reliable results could be obtained.

ENGLAND.—The investigation was discontinued for some time, but when working in England upon the effect of light polarized by a Nicol on the leaves of sow-thistle, attention was again directed to it.

A leaf shaded from the setting sun, picked in the evening of a cloudless day, gave very little starch, but when a bright morning



FIGS. 10, 11.—Leaves from patches of lucerne: fig. 10, exposed to afternoon polarized light; fig. 11, from adjoining patch kept in darkness.

was followed by a dull afternoon with slight rain in the early evening, in a similarly situated leaf the full starch content remained. This could only be explained by concluding that in the first case the starch had been hydrolyzed by the clear blue skylight (which on testing was found to be strongly polarized), while in the second the light was depolarized by cloud and moisture, and thus no hydrolysis took place.

SOUTH AFRICA.—In carrying out the experiments upon spinach leaves already described, it was noted that when the leaf was placed in such a position as to receive skylight from the area of polarization, but was shaded from the sun, that part of the leaf exposed to daylight had less starch than the part under the wide rim of the

Nicol's prism, which was in darkness. As a consequence of these preliminary results, series of investigations were undertaken and photographs of some of the results are given (figs 10-15).

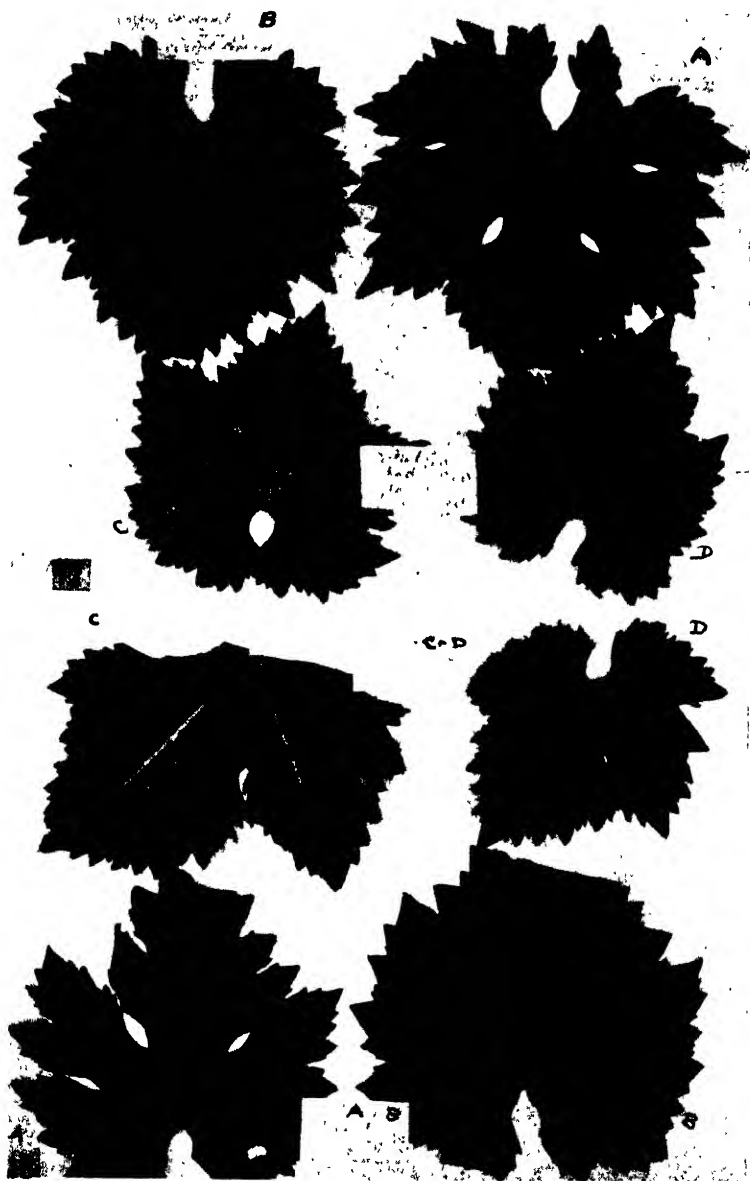
FIRST SERIES

Small patches of young lucerne plants, growing on the ground and of the same age and appearance, were chosen, so that one (fig. 10 *P*) could be exposed to the afternoon skylight in that part of the sky having maximum polarization, but shielded from the sunlight, while the other (fig. 11 *DK*) was lightly covered by a thin wooden box, giving ample admission of air. The temperature was noted under such conditions to be exactly the same in the two cases. The experiment was started at 4:00 P.M., and the two sets of leaves were picked at 6:00 P.M., choosing those leaves which stood out best to receive both the mid-day light and in one case the afternoon light. The experiment was repeated several times with similar effects. On one occasion the sky became cloudy and then no difference was observed, as the light was only feebly polarized.

SECOND SERIES

Most interesting results were obtained with vine leaves. In the College grounds are two rows of stone pillars, about 2 feet square, round which large vines are trained. Leaves were chosen growing on the east side of these pillars, these being shielded from the afternoon sun after 1:00 P.M. but pointing to that part of the sky at which the average polarization was greatest. All the leaves received full sunlight up till 1:00 P.M.

Experiment I (fig. 12).—Four of the freshest leaves were chosen: *A* was picked at 4:00 P.M., having received only morning and early afternoon light; *B* was partly covered by a thin dark envelope, leaving the lobe *P* exposed; *C* and *D* were left fully exposed to the blue polarized skylight till sunset at 7:00 P.M. On staining with iodine *A*, which had 3 hours less daylight than *C* and *D*, had full starch while the latter showed faint purple only at the edge. The partly covered leaf showed less starch in the exposed part than in the part in darkness. (The line of demarcation is not sharp, because the envelope was only lightly attached, to give free play of air to the surface of the leaf.)



FIGS. 12, 13.—Fig. 12, vine leaves: *A*, picked 4:00 P.M. after bright morning; *B*, right leaf half-covered, lobe *P* being left exposed 4:00–7:00 P.M.; *C*, *D*, picked 7:00 P.M., having had 3 hours more bright skylight than *A*; fig. 13, *A*, *B*, picked at 2:30 P.M.; *C*, *D*, picked at 7:00 P.M., having had $4\frac{1}{2}$ hours more skylight than *A* and *B*.

Experiment II (fig. 13).—In this case *A* and *B* were picked at 2:30 P.M. and showed full starch content; *C* and *D* were left till sunset and were almost devoid of starch. (*C* was a large leaf and there was only room for part of it. A large leaf was chosen together with a small one, to show that size was not the determining factor.)

THIRD SERIES

Experiments upon *Tropaeolum* were equally interesting. Two pots of young plants of the same age and size were taken. One was lightly covered from 4:00 to 6:00 P.M., the other being exposed to polarized skylight (*P*). The most prominent leaves were picked and treated with alcohol and iodine.

The results are seen in figs. 14 and 15. Again the polarized light of the sky caused hydrolysis, while in darkness the starch remained practically unchanged.

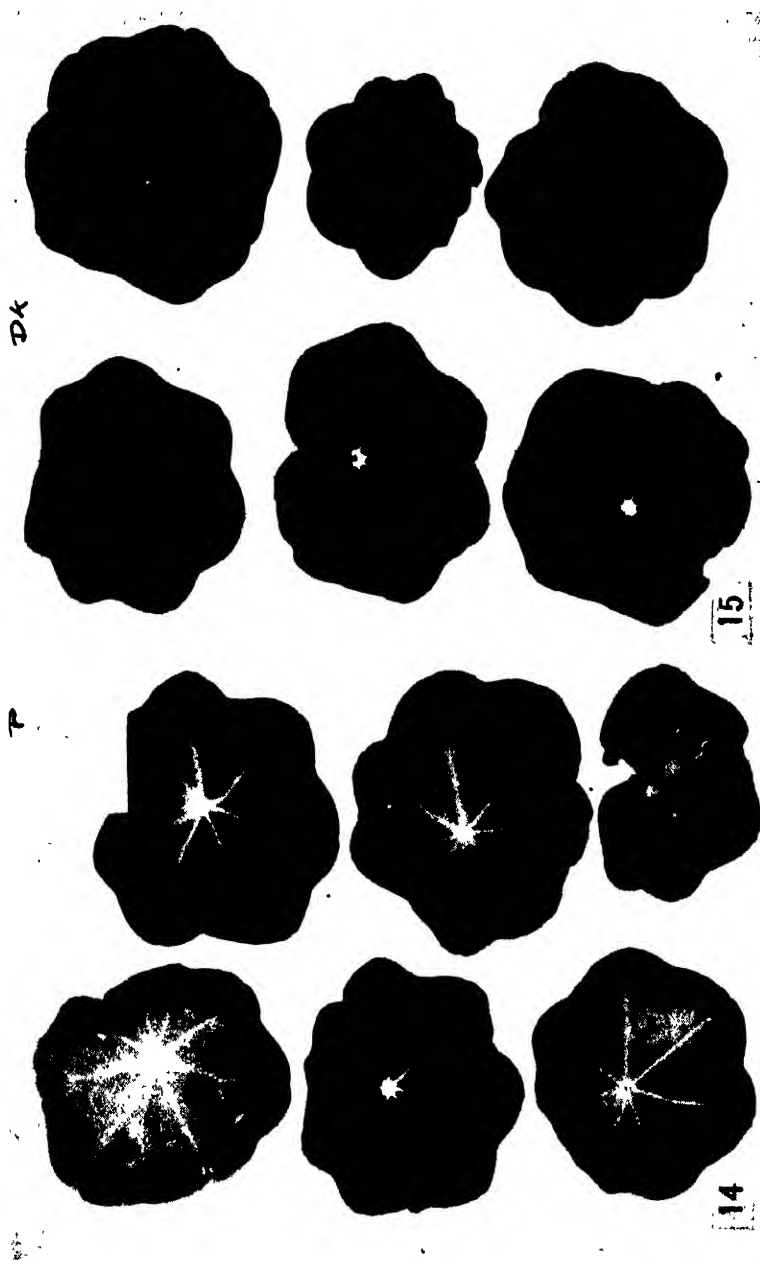
Result

The invariable result of the numerous experiments performed, only a few of which are here reported, must lead to the conclusion that starch, which is formed in the sunlight and non-polarized daylight, is digested by the polarized light of the late afternoon (and early morning), especially when the sky is clear. In cloudy weather it is probable that the chief element is the enzyme of the leaf, which acts slowly in the dark and is dependent on temperature. The remarkable acceleration of the process of digestion produced by polarized skylight must greatly increase the rate of growth of the plant. However plentiful the food supply, a living organism can only use that which it digests.

III. Effect upon growth of whole plant

It is evident that the hydrolysis resulting from polarized light must have an important bearing on the growth of the whole plant. One or two simple experiments have been made in this direction, which may be of interest in paving the way for further systematic investigation.

As a simple test, a young plant was placed on a laboratory bench so that it received only light polarized by a polished reflecting surface, the direct light from the window being excluded. Although sufficiently watered, the plant died in about a fortnight. As an in-



FIGS. 14, 15.—Fig. 14, *Tropaeolum*, exposed to polarized skylight 4:00–6:00 P.M.; fig. 15, from adjoining pot, covered 4:00–6:00 P.M.

quiry into the cause of such death the following experiment was arranged.

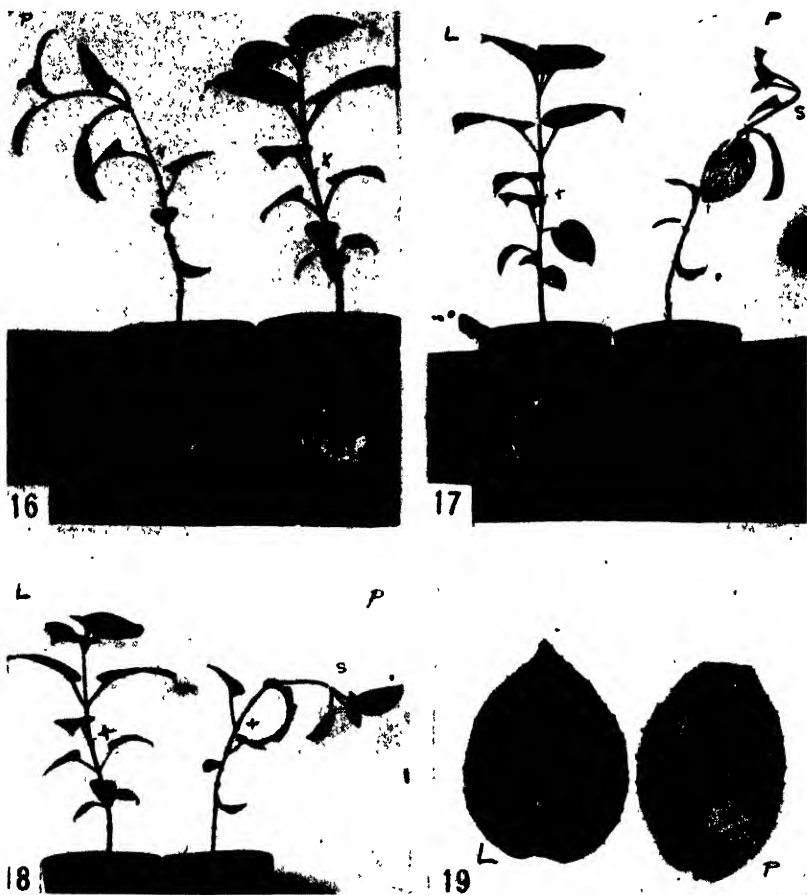
A long box, divided into separate compartments, was placed on its long side in front of a north window (N by NE). The backs of these compartments were formed by sheets of wood tilted at an angle of 53° with the horizon. The back of the polarizing chamber was covered by ferrotype, upon which was a sheet of glass. The light from the window was thus polarized by the polished surface and reflected vertically downward upon the plant within the chamber. The back of the control chamber was covered with a piece of dull rough paper, which could be changed to increase or diminish the brightness of this diffused non-polarized light. The direct light from the window was intercepted by a screen in front of the lower half of the two chambers. Plant *P*, therefore, in the polarizing chamber, received a diurnal succession of polarized light and darkness, while plant *L* in the control chamber experienced ordinary light, alternated with darkness. Intensity of the light was tested by photographic paper, placed in similar positions within the two chambers, and was found to remain remarkably equal.

Equal amounts of water measured with a pipette were given to each plant daily.

Result

Both plants, which were chosen by an expert as being similar in height, number of leaves, and healthy appearance, remained in full vigor during the first two days. *P* looked somewhat more vigorous, as its digestion was being wonderfully improved. Soon it began to weaken and show signs of starvation, however, and the extraordinary contortions shown in figs. 16-18 were produced by the hydrolyzed products of the leaf passing down one side of the stem, causing increased turgor on that side. This swelling can be seen on the stem at *S*. The plant was placed so that the polarized light fell full on one or two leaves and the stem turned away from the polarized light. When the pot was turned round so that the inclination was now toward the polarizing surface, the plant persistently turned away again. This occurred most strongly at midday, when the light was brightest. The deflection at one time amounted to about 90° in 1.5.

hours. In the darkness it recovered, and in the early morning the stem was found again to be erect.



FIGS. 16-19.—Figs. 16-18, contortions of stem of young fuchsias, owing to passage of products of hydrolysis down the stem; in each case plant turned away from direction of polarized light; fig. 19, *P*, disappearance of starch and attenuation of leaf tissue of plant exposed in polarizing chamber; *L*, from control in ordinary diffused light, here starch remained and leaf grew healthily.

A leaf was picked from each plant, the position being similar (marked +), and tested for starch. With iodine the control gave a clear purple color, while *P* (fig. 19) was not only practically devoid of starch, but was thin and emaciated.

This corresponds with the fact noted in part I, that where long exposure of the leaf was made behind a Nicol's prism the leaf tissue itself broke down. The experiment was continued for several weeks, and gradually the leaves on *P* dropped off, leaving only two at the apex. The plant was found bending completely over to the ground, but on taking it out of the polarizing chamber and placing it near the laboratory window in ordinary diffused skylight, it recovered its erect posture and began to show signs of returning health.

The control maintained throughout a steady growth and an almost erect position (figs 16-18). Both plants contained an amount of anthocyanin in the young stems. This disappeared in plant *P* but remained in the control. This fact is of interest when compared with the result described in part I, where the anthocyanin in a leaf of *Beta vulgaris* was hydrolyzed to anthocyanidin by exposure for several hours behind a Nicol's prism. This same absence of anthocyanin was observed in another experiment, where mustard seedlings were grown under polarized light, although it was present in the control in ordinary light.

Summary

Plants grown in polarized light alone, succeeded by periods of darkness, exhibit the following results: (1) disappearance of starch and other reserve products, such as glucosides, etc.; (2) temporary phototropism (usually negative, so-called) owing to increased turgor in stem; (3) leaf fall and signs of starvation in whole plant. The second of these effects will, of course, depend on the morphology of the stem and leaf.

The inference is evident, and is in full accord with the interesting observations upon the growth of seedlings by MACHT.³ For rapid and healthy growth the plant requires the alternation of polarized and non-polarized light, such as is provided by nature on a bright summer day.

I am greatly indebted to the authorities of Bedford College, London University and of the Huguenot University College, South Africa, for granting me facilities for this research. I heartily thank

³ MACHT, D. I., Influence of polarized light on the growth of seedlings. Jour. Gen. Phys. 1: 41-52. 1925.

President BERTHA STONEMAN for her valuable advice and assistance in the investigation. I am also indebted to Dr. FRANK THONE, I should like also to tender thanks to Mr. F. T. LACEY, and Miss WILLIAMS of the Chelsea Polytechnic, and also Mr. HALES of the Chelsea Physic Garden for much help and provision of facilities in the early stages of this work. Lastly, I express great appreciation of the help given me by the late Dr. E. E. SLOSSON.

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BARK STRUCTURE OF CALLIXYLON¹

CHESTER A. ARNOLD

(WITH SIX FIGURES)

Some details of bark structure are shown in a few specimens of *Callixylon* wood recently collected from the Genundewa limestone horizon at Bristol Center in Ontario County, New York. Although not well preserved and fragmentary, these specimens are of considerable interest because of our scant knowledge of the phloem and cortex of Devonian plants in general. This lack of knowledge is especially true of the phloem and the inner portion of the cortex. The outer cortex, which frequently contains strands of sclerenchymatous fibers, is often found in connection with petrified stems, but usually all structures between this and the wood have completely disappeared.

Several years ago PENHALLOW² described and figured the bark of a specimen from about the same locality and horizon as the material dealt with in this account. This specimen, which he described as *Cordaites hamiltonense*, was so poorly preserved that an accurate description of the wood was not made. His figures of the bark structure correspond closely to the material described in this account, and because of the abundance of *Callixylon* wood and the almost complete absence of other types in that region of New York, it is probable that he was working with this form also.

In this account the term "bark" is used as an inclusive designation of all the tissues concerned, since it is impossible to distinguish clearly the cortex from the phloem in the material at hand. Because of its proximity to the xylem, the inner portion of the bark at least is certainly phloem, but the slight differentiation of the tissues farther out is not sufficient to mark a boundary between the phloem and the cortex. Moreover, it is barely possible that the entire structure is phloem, the cortex not being present.

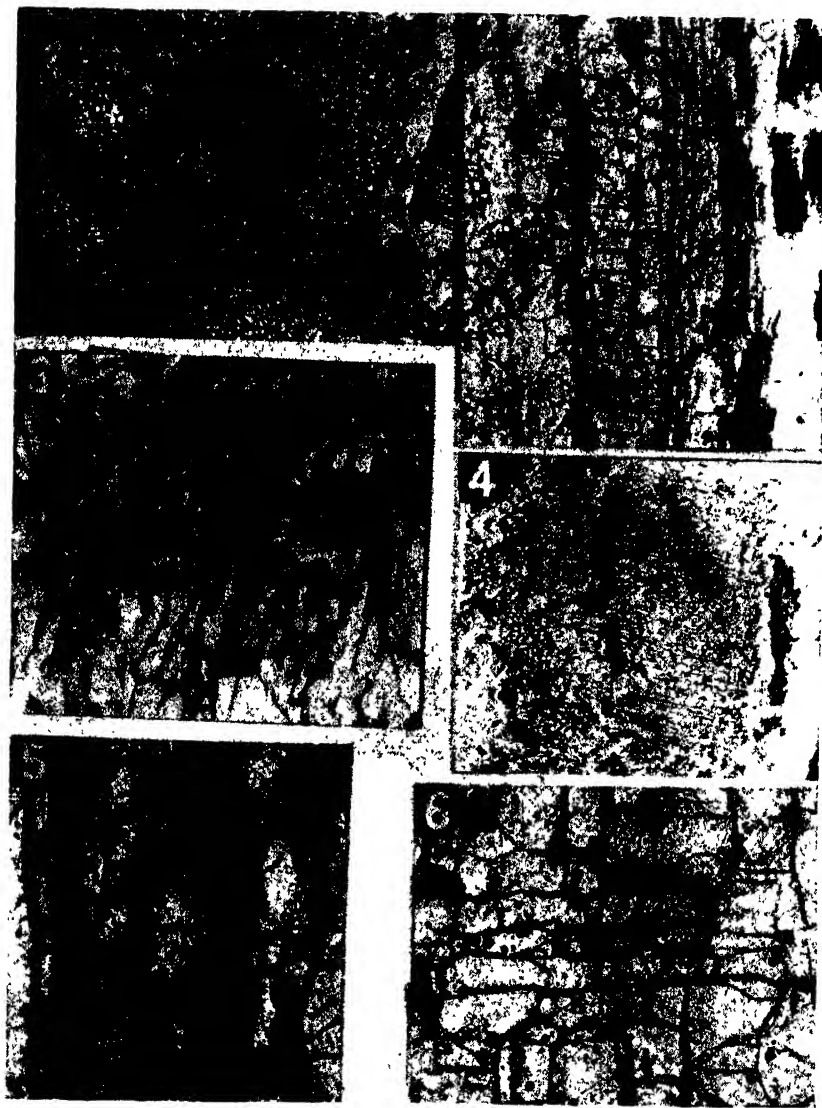
¹ Paper from the Department of Botany of the University of Michigan, no. 327.

² PENHALLOW, D. P., Notes on the North American species of *Dadoxylon*. Trans. Roy. Soc. Canada 6 (4):51-77. 1900.

This study was made entirely from radial and tangential sections. There was sufficient material for several sections, but all the tangential cuts were through portions too poorly preserved to be studied satisfactorily. In all instances the amount of bark preserved was small. The material consists of the woody axis of a root 9 mm. in diameter, bearing a bark fragment 3 mm. thick, and a stem 3.5 cm. in diameter, embraced in about one-fourth of its circumference by a bark layer 2 mm. thick. In the latter specimen the bark and wood were separated by an empty space about 3 mm. wide. That this space was caused by a forcing apart of the wood and bark during crystallization of the petrifying agent and not by decay of intervening tissues is indicated by fragments of phloem still in contact with the wood, the splitting having taken place in some instances a short distance from the cambium.

The preserved portion of the bark of the root, in addition to being thicker than that of the stem, shows some structural differences probably associated with the age of the specimens. Adjacent to the xylem is a thin, partially crushed layer of radially arranged cells (not shown in illustration) which was beyond doubt the functional phloem. Just outside this, where preservation is better, the tissue is made up largely of thin walled empty cells, and scattered throughout are clusters made up of few to twenty or thirty cells with a dark content which was probably originally tannin (fig. 1). In cross-section the dark cells are nearly square and measure about $30\ \mu$ in diameter. The empty cells are in two general types: some of them are elongated radially and others have their transverse and radial diameters about equal. In the outer portion of the bark they become more irregular in size and arrangement, the radial elongation becomes less apparent, and some of them become of greater diameter tangentially than radially.

In the stem, which was older than the root, a slightly different arrangement is apparent. The tissue of empty cells alternates in a radial direction with layers of tannin cells, the latter being two to five cells in radial extent (fig. 4). The continuity of these bands is frequently interrupted by small groups of empty cells (fig. 3). Throughout the empty celled tissue between these bands of tannin cells are a few scattered tannin cells, either occurring singly or in



FIGS. 1-6.—Fig. 1, transverse section through bark of *Callixylon* roots showing scattered groups of tannin cells, $\times 17$; fig. 2, radial section through bark of stem, $\times 35$; fig. 3, portion from near center of fig. 4 but more highly magnified, $\times 70$; fig. 4, transverse section through bark of stem showing two types of empty cells and banded arrangement of tannin cells as contrasted with scattered arrangement in root, $\times 17$; fig. 5, portion of radial section of phloem similar to fig. 2 but more highly magnified, showing two types of empty cells and their relation to tannin cells, $\times 115$; fig. 6, portion of phloem ray, $\times 115$.

groups of two or three (figs. 3, 4). The tannin cells, when viewed longitudinally, are several times as long as broad and appear to have oblique end walls. As in the root, there are two types of empty cells: those with radial and vertical diameters about equal but shortened tangentially, and others having radial and shortest tangential diameters about equal but exceeded three or four times by the length (figs. 2-5). The latter type of cell seems to occur mostly in close proximity to the tannin cells. Other thin walled cells in which the greatest dimension is radial are so arranged as to stimulate phloem rays (fig. 6). In transverse section these rays are not readily distinguishable but they are conspicuous in radial section.

Structures resembling sieve plates are not distinguished with certainty, and all the cells except possibly the tannin cells have transverse end walls. As already stated, it is uncertain whether this tissue is phloem or cortex except for its proximity to the xylem and for the structures which appear to be phloem rays. It is possible that a considerable quantity of it is old phloem which has undergone alteration, but the younger and functional phloem is small in amount and not well preserved.

Unlike the secondary xylem of *Callixylon* the phloem (assuming that it is phloem) shows comparatively little differentiation. Some of the inner poorly preserved portions show a radial arrangement indicative of cambial origin, so this form scarcely supports the contention of some investigators that the early vascular plants were without true phloem. The scarcity of phloem in vascular plants is generally due to its destructible nature and its location on the outside of the wood axis where it is subject to various destructive forces.

As stated, PENHALLOW'S *Cordaitea hamiltonense* (which is probably *Callixylon*) shows bark structure similar to that just described. He says that the "resin canals" (tannin cells) are devoid of transverse septa, but he probably failed to observe them as they are obscure and difficult to see.

In *Mesoxylon sutcliffii*, a cordaitean species whose phloem is described by MASLEN,³ a more complex organization exists, a distinct pericyclic zone separating phloem from cortex. The cortex shows

³ MASLEN, A. J., The structure of *Mesoxylon sutcliffii* Scott. Ann. Botany 25:381-412. 1911.

pronounced periderm formation which, if it existed in *Callixylon*, is not preserved. The phloem contains vertically elongated cells with dark contents apparently very similar to those in *Callixylon*, but the rays and the radial arrangement of the elements are more pronounced.

Since *Callixylon* is Upper Devonian and *Mesoxylon* is from the Lower Coal Measures it is especially interesting to find the simplest bark structure in the older form. The same cannot be said of the secondary wood. Although *Callixylon* is one of the oldest known members of the cordaitean complex, the radial arrangement of the pit groups in the wood appears to be a highly specialized feature. Also there is an enormous development of secondary wood, as shown by the recent discovery of trunks 3 feet in diameter and several feet in length. The cryptogamic primary wood, which is similar to that of *Pitys*, probably places it below the true cordaiteans in the evolutionary scale. The discovery, then, of the rather simple phloem and cortex, although fragmentary and small in amount, suggests that *Callixylon* was a rather primitive plant in which development of the secondary wood was probably out of proportion to that of the other tissues.

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CURRENT LITERATURE

BOOK REVIEWS

Recent investigations of Devonian plants

A valuable contribution to our knowledge of American Devonian plant structures is a doctor's thesis¹ which has been prepared at Cornell University. The material was obtained from a region in the west central portion of the state of New York, primarily from the Genundewa limestone, but also from the Genesee shale which underlies the Genundewa limestone, and from the Portage group, which is above the limestone. The three horizons belong to the Upper Devonian. The preservation in which the plant material appeared is that of wood fossilized by saturation with calcium carbonate; also preservation by iron sulphide (either pyrite or marcasite) occurs. This fossil wood belongs to the genus *Callixylon*, which was founded by ZALESSKY in 1911 on some material from the Upper Devonian of the Donetz basin, but later was found in the Devonian of Ohio and described by DAWSON, PENHALLOW, ELKINS and WIELAND, and HYLANDER.

ARNOLD made thin sections from twenty-four different specimens, seventeen of which are mentioned in his paper. He establishes four new species. *Callixylon zaleskyi*, *C. mentethense*, *C. erianum*, and *C. bristolense*. ARNOLD shows that *Callixylon* was one of the dominant types of the Upper Devonian period. It had already a high degree of specialization, and cannot be considered as primitive in spite of its mesarch primary wood. It distinguishes itself by its grouped pitting from Cordaites, which had continuous pitting. *Callixylon* is characterized by the absence of leaf gaps, and it appears that this genus represents the termination of a very ancient group of plants from which Pteropsida developed at a very early period. The occurrence of scattered medullary tracheids in *Callixylon* would suggest its origin from a previous protostelic complex. *Callixylon* must have been a highly organized plant which probably flourished during the Upper Devonian to the same extent that Cordaites did during the Carboniferous. It was probably not in the line of direct descent of later forms, but reached its climax and disappeared at the end of the Devonian or early Mississippian. It probably sprang from a stock common to both the Pteridospermeae and the Cordaitales.

ARNOLD's valuable investigation supports SCOTT's statement that *Callixylon* is the most highly differentiated wood structure, and is also the oldest member of

¹ ARNOLD, C. A., The genus *Callixylon* from the Upper Devonian of central and western New York. pp. 50. pls. 19. 1 map. Reprinted from Papers Michigan Acad. Sci., Arts & Letters. Vol. XI. 1929. Published 1930.

the Pityeae. It is a diversified genus, terminating a pronounced evolutionary process which was either gradual and long or intense over a shorter period. Whether it was pteridospermous or cordaitan or something different is a question to be settled only on the acquisition of more information.

To the series of contributions to the study of the Devonian floras by KRÄUSEL and WEYLAND² has been added a third instalment. The third number of the series contains a description of the following species: *Asteroxylon elberfeldense* Kräusel and Weyland, *Aneurophyton germanicum* K & W, *Hyenia elegans* K & W, *Calamophyton primaevum* K & W, *Cladoxylon scoparium* K & W, *Protolepidodendron scharyanum* Pot. & Bern., *Haspia devonica* K & W, *Kicklingia erecta* K & W; and a new genus, *Duisbergia* K & W. A number of spores and sporangia and several types of wood showing structure are also discussed.

The authors claim that the Devonian plants are a key for a morphogenetic understanding of the higher plants; they represent types from which all the others can be derived. They assume that these Devonian plants represent the oldest known vascular plants, but those plants already are very far removed from the age of thallophytes.

This number of the series contains 43 pages of quarto and 15 plates. It is to be hoped that the authors will continue with these valuable contributions to our knowledge of the Devonian floras.—A. C. NOÉ.

Physiology and biochemistry of bacteria

The second and third volumes of BUCHANAN and FULMER'S³ monumental work on the physiology and biochemistry of the bacteria have been published. The first volume of this treatise was noted⁴ two years ago. The chapters in the three volumes are numbered consecutively, although the volumes are each pagged separately. Volume II covers the effects of environment on organisms, and contains chapters vi-xiii, while volume III, the effects of microorganisms on the environment, contains chapters xiv-xix. This last volume is particularly valuable to students of anaerobic respiration and fermentations.

The discussion is organized into five sections, A, B, and C in volume II, and D and E (mislabelled A and B) in volume III. Section A presents the problems of recognition and measurement of the effects of environment on microorganisms. Section B discusses the effects of physical factors in the environment on bacterial and other microbial life. Section C deals with the effects of chemical factors of the environment. Section D considers special physiological interrelations of mi-

² KRÄUSEL, R., and WEYLAND, H., Beiträge zur Kenntnis der Devonflora. Frankfurt. Senckenbergische Naturforschende Gesellschaft. I. 1923. II. 1926. III. 1929.

³ BUCHANAN, R. E., and FULMER, E. I., Physiology and biochemistry of bacteria. Vol. II. Effects of environment upon microorganisms. 8vo. pp. xviii+709. Vol. III. Effects of microorganisms upon environment. Fermentative and other changes produced. 8vo. pp. xvi+575. Williams and Wilkins. Baltimore. 1930.

⁴ Bot. Gaz. 86:1118. 1928.

croorganisms, particularly antagonisms, commensalism, and symbiotic relationships of many kinds. The first chapter in the final section, E, deals with the chemical agents of decomposition, the enzymes, including hydrolases and anhydases, esterases, deaminases, reducing and oxidizing enzymes, etc. This is an important chapter to students of general physiology. There follows a general discussion of chemical changes produced by microorganisms in their environment, and then a more specific discussion of the changes produced in inorganic (carbonless) compounds; changes in the non-nitrogenous organic compounds; and in the nitrogenous constituents of the environment.

In each volume the literature lists and indices occupy 150-170 pages. The three volumes together total 1800 pages. It is difficult to overstate the value of such a work. It is a great storehouse of information, bringing together an immense and inclusive literature which is inaccessible to the ordinary student because it is so widely scattered, and because the process of locating and consulting the original works is so time-consuming.

The authors deserve great praise for their willingness to perform such a service to the science of bacteriology and physiology. In some places the material does not seem to be as well digested as it should be, but it is an extremely difficult task to evaluate the contributions over so vast a field, and draw them together into a well balanced, critical review. The work will prove extremely useful, and should stimulate work by bringing into relief conflicting views of bacterial physiology. It ought to meet the approval and appreciation of a large group of scientists and scientific organizations.—C. A. SHULL.

Inheritance in fungi

A timely and welcome contribution to genetic literature has been supplied by KNIEP.⁵ A competent summary of the genetic data relative to the fungi has been needed ever since it was demonstrated for some fungi that they, and therefore all fungi, probably do not stand apart from other plants in their mechanism of inheritance. The observations that in many fungi there is an extensive haplophase which can be cultured and observed as an independent organism, that heterozygosis occurs in many cases and that it can be experimentally manipulated in some, provide instruments for direct demonstration of segregation of genes. The author restricts himself to a discussion of those instances of variability of fungi in which characters are involved that are manifest in the genotype and are passed through the zygote to the progeny, thus eliminating most of the cases which have been interpreted and reported as mutations.

The subject matter is subsumed under (A) chromosome inheritance, which constitutes the major part of the discussion; and (B) plasmatic inheritance, a brief discussion which interprets GOLDSCHMIDT's studies on *Ustilago violacea* and HARDER's merogony experiments with *Pholiota mutabilis* as giving a basis for assuming plasmatic inheritance in fungi. The major portion of the discussion of

⁵ KNIEP, H., Vererbungserscheinungen bei Pilzen. Bibliographia Genetica V. pp. 371-478. figs. 15. Martinus Nijhoff. Gravehage. 1929.

chromosome inheritance is devoted to the phenomena of sex inheritance, and the remainder to inheritance of vegetative characters.

There is a comprehensive bibliography and a detailed index which add greatly to the usefulness of the volume.

The presentation is clear and critical. Its intelligibility is enhanced by illustrations, diagrams, and tables, many of which are original. The reader will find the free use of well chosen illustrations a great aid in the discussion of DODGE's interesting studies of the genetics of *Neurospora sitophila*.

The volume is marked by the same mastery of facts and fertility of ideas which characterized the author's preceding volume, *Die Sexualität der niederen Pflanzen*.—G. K. K. LINK.

Elementary course in general physiology

A textbook and laboratory manual combined in a single volume has been prepared by SCARTH and LLOYD⁶ of McGill University. In order to bring out the similarities and differences between cells and physical and chemical systems, the chapters are arranged more or less in pairs, as indicated by the following chapter headings: Life as a mechanism; Organization of protoplasm; Cell structure; Surface tension in physical systems; Surface tension in cells; Adsorption in physical systems; Adsorption in cells; Diffusion and osmosis in physical systems; Diffusion and osmosis in cells; Ions and their determination in physical systems; Ions, particularly H-ions, and their determination in cells; Electric potential and electric currents in physical (non-metallic) systems; Electric potential and electric currents in cells; Colloids in physical systems; Colloids in the cell. These chapters occupy 190 pages of text. Part I deals with principles and theory; part II presents a laboratory outline, running more or less parallel to the text material. The last two outlines deal with ultramicroscopy and enzymes.

The book will prove suggestive to many who have wanted to bring into the laboratory work in biology something of the flavor and technique of physical chemistry and physics. It is not possible to include everything one might desire in so small a space, of course, but the authors have illuminated many of the processes of living cells for the student who is making his first attempts at understanding the processes of organized living beings.—C. A. SHULL.

Number 9 of volume I of *Die Pilze Mitteleuropas*⁷ has appeared. It is a continuation of the Boletaceae, and presents *Boletus parasiticus* and *B. lignicola* in two colored plates. In addition, *B. sulphureus* is illustrated in color in one of these plates. Plates in black and white illustrate *B. luteus*, *B. bovinus*, *B. variegatus*, *B. parasiticus*, and *B. lignicola*. The text is devoted to *B. parasiticus* and *B. lignicola*.—G. K. K. LINK.

⁶ SCARTH, G. W., and LLOYD, F. E., Elementary course in general physiology. 8vo. pp. xxii+258. John Wiley and Sons. New York. 1930.

⁷ Die Pilze Mitteleuropas, under the editorship of KNIPE, H. (Berlin); CLAUSSEN, P. (Marburg); and BASZ, J. (Stuttgart). W. Klinghardt. Leipzig. 1928.

Microbiology

A volume on microbiology by LUTMAN⁸ differs from other textbooks which cover essentially the same subject matter, that is, the morphology and physiology of bacteria and fungi and the significance of these in the economy of nature and in the lives of humans. Unlike textbooks in bacteriology in which usually only passing attention is given to the fungi (yeasts and "molds"), and textbooks in mycology in which no or the merest mention is made of bacteria, this volume treats both of these assemblages of organisms so that a sense of their relatedness and equal importance is gained, even though more chapters and pages are devoted to bacteria than to fungi. This is accomplished by organization of the general physiological aspects of microorganisms about types which include such fungi as *Rhizopus nigricans* and other phycomycetous allies, and *Saccharomyces* sp., *Penicillium* sp., *Aspergillus* sp., together with such ascomycetous allies as *Sclerotinia cinerea*, the rotter of fruits in the orchard, transit, storage. In these chapters the phenomena of growth and invasion of other organisms are discussed in their relationship to temperature, humidity, and soil factors, as well as the phenomena of digestion and respiration in microorganisms. Naturally fermentation receives considerable attention.

The author has found it necessary to deviate from the type method of presentation, and to use some general chapters. The beginning chapters present definitions and concepts, historical matter, and practical laboratory matters, while the following chapters present theories, problems, difficulties, and controversies that confront the modern microbiologist. Thus two of the closing chapters are devoted to the problem of constancy of species.

It is a sane, well balanced, informative, and stimulating presentation of one of the most fascinating and practically significant aspects of biology.—G. K. K. LINK.

Grass manual

The Indiana Department of Conservation has again taken advantage of its opportunity by publishing another of DEAM's books on Indiana plants. This volume⁹ is a companion to the earlier publications of DEAM.¹⁰

The arrangement of the tribes and genera is that employed by HITCHCOCK in his *Genera of grasses in the United States*, which places the less specialized groups first. The treatment includes dichotomous keys to the genera of each tribe, while each genus has a key to species. Rather complete botanical descriptions have been drawn by the author from Indiana material. The general dis-

⁸ LUTMAN, B. F., Microbiology. pp. x+495. figs. 211. McGraw-Hill Book Co. New York. 1929.

⁹ DEAM, C. C., Grasses of Indiana. Publ. 82. Dept. Conservation, State of Indiana. 1929.

¹⁰ ———, Trees of Indiana. Publ. 13. Dept. Conservation, State of Indiana. 1921.

———, Shrubs of Indiana. Publ. 44. Dept. Conservation, State of Indiana. 1924.

tribution of species is described briefly. In addition the distribution of each species within the state is indicated by counties on separate maps by use of initials of the herbaria in which the specimens are deposited.

The excellent illustrations have been prepared by PAUL WEATHERWAX, who has also contributed an adequate account of the morphology of the grass plant. In each genus the habit of at least one species is illustrated, and the spikelet of each species is figured. The size and detail of the plates add considerable facility to the use of the manual, especially in view of the fact that the botanist usually avoids identification of grasses. The work recognizes 163 native species, 16 varieties, and 7 forms; 38 introduced species, and 3 varieties. For various reasons DEAM excludes 51 species that have been reported as occurring in Indiana.—S. A. CAIN.

NOTES FOR STUDENTS

Control of seeding behavior.—The causes of premature seeding of celery have been investigated by THOMPSON,¹¹ who finds that hardening of the seedling plants at low temperature (40–50° F.) is the main cause of this behavior. Freezing itself, popularly believed to induce first season blooming, was found to retard rather than to hasten it. Prolonged cold is particularly effective. With only 7–10 days of chilling the plants usually do not seed during the first summer, but remain biennial; with two weeks' treatment or longer, however, the plants are likely to show premature development of the reproductive phases of the life cycle, and become annuals. Hardening of the plants in the cold-frame by low temperature for too long a time is the most potent cause of commercial loss from this physiological disturbance.

Since the plants can be hardened effectively by water deficiency, without use of low temperatures, it is easy to prevent premature seeding. When late celery is seeded in outdoor beds, it may be induced to form seed stalks by cool weather during the seedling stages. (The reviewer has had the same experience with early sown turnips.) But high temperatures (70° F. or above) later in June and July can overcome this tendency, and prevent the premature development of blossoms.

High temperature treatment will prevent seeding, regardless of the previous treatment, provided differentiation has not proceeded too far before the high temperature is applied. Such facts as these account for the irregular behavior of celery under commercial cultural conditions. Early cold weather may be offset later by favorable high temperature conditions.

Naturally there are varietal differences in behavior. The rank-growing early sorts are more susceptible to climatic modification. The strains inherit certain capacities for development along vegetative and sexual lines. But the environmental conditions determine the exact nature of the response, developing vege-

¹¹ THOMPSON, H. C., Premature seeding of celery. Cornell Univ. Agric. Exp. Sta. Bull. 480. 1929.

tative or reproductive responses mainly through temperature modifications of metabolism.

As in many other such studies, chemical analyses of the plants do not help much in interpretation of the behavior. This is probably due to the fact that the effective metabolic conditions are centered in the differentiating region of the stem tip meristem, while our analyses are often made from the entire shoot. The differences in meristems may then be outweighed by the general average condition of the older tissues. Microchemical differences in the heart regions of these plants should be sought, and at the time differentiation is occurring, rather than after the differentiated plants have grown under the same conditions as the controls for a long period.

A study of seed stalk development of cabbage has been made by MILLER,¹² who also finds that temperature plays a large part in determining the vegetative and sexual responses of cabbage plants. Stalks of cabbage in head, removed to a warm greenhouse (60–70° F.), remained vegetative for two and one-half years, producing head after head, with intervening periods of stem elongation. After producing six successive heads on the same stem it was removed to a cool greenhouse, under which condition it blossomed in a few months. Heads given a rest period of two months in cold storage (40° F.) bloomed a month earlier in the cool greenhouse than those not given such a rest period. After such cold storage treatment, high temperature hastened seed development instead of inhibiting it. Cool treatment in the young stages of development was very effective in inducing seeding.

Chemical analyses of the older plants were made on samples from the heart region, and showed a definite accumulation of carbohydrates in the seeders. Analyses of the young plants showed little. Here again microchemical methods might be more effective.

As in the case of celery, it is a problem of both heredity and environment, a capacity to respond in various ways, and development of one response rather than another by the surrounding conditions, through some profound influence upon physiological processes and metabolism, particularly as these affect differentiation.

These papers are excellent contributions to our knowledge of plant behavior, and are helpful in pushing toward the goal of physiological research, controlled plant production.—C. A. SHULL.

¹² MILLER, J. C., A study of some factors affecting seed-stalk development in cabbage. Cornell Univ. Agric. Exp. Sta. Bull. 488. 1929.

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